Use And Selection Of Terrestrial Resources By Adult Common Toads (*Bufo bufo*) In Agricultural Landscapes

By
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ABSTRACT

Radio-tracking was used to determine habitat use and preference. Implantation of transmitters resulted in a significant reduction of feeding in female but not in male *B. bufo.* Tagging by ingestion caused reduction in feeding when initial body mass of *B. bufo* was below 25g and tag mass was 2.5g. Therefore ingested transmitters were used to radio-track 103 *B. bufo*, of mass > 25g, between May-September of 1993 - 1995. A hand -held portable radio-tracking system was used to track *B. bufo* within a 0.1 hectare enclosure, managed to provide four commonly occurring habitats. *B. bufo* whether active or in refugia, demonstrated a significant preference for woodland and rough grassland by comparison with arable and pasture. These preferences were less evident in a humid summer, especially for males.

The food available to toads within the radio-tracking area was monitored using pitfall trapping and suction sampling. The stomach contents of 128 *B. bufo* were collected during May-September 1993 to 1995 and analysis were weighted to reflect extent of digestion. Arable had a significantly higher dry mass and abundance of Coleoptera than other habitats and dry mass of Coleoptera was higher in all habitats in a humid summer. Nocturnal pitfall traps had significantly fewer small invertebrates (<2mm) than diel traps. The diet of *B. bufo* was dominated in terms of both abundance and dry mass by Coleoptera. The small taxa such as, Acari and Collembola, had the most negative electivity indices. Coleoptera had the strongest positive electivity index for both diel and nocturnal prey availability data. *B. bufo* which did occupy arable+pasture habitats had a higher mass of Coleoptera in the diet compared with those from rough grassland+woodland.

The microclimates of woodland and long grassland ameliorated the temperature and humidity fluctuations recorded in arable and pasture. Microclimates of refugia had smaller diurnal variations in temperature and humidity than the microclimates of surface habitats. Survival and mass loss of 162 *B. bufo*, in nine habitat specific enclosures were recorded along with enclosure microclimate. Despite surviving losses of up to 58.6% of body mass, *B. bufo* in short grassland had significantly reduced survival and higher mass loss (correlated with minimum humidity) than *B. bufo* in woodland or long grassland enclosures.

Adult *B. bufo* were counted at 26 ponds during two breeding seasons and the counts were corrected for water turbidity. In the intervening autumn the 1km radius around each breeding site was assigned to broad habitat categories which were compared to the population counts using multiple linear regression. The area of long grassland was significantly positively correlated with the population counts of both male and female *B. bufo* in both years. Based on these regressions, a minimum viable population of 30 females would be sustained by, between 6 and 11 hectares of long grassland.

Toads in an agricultural landscape, with a mosaic of habitats, were able to move between areas of high prey availability, despite potentially desiccating microclimates, when macro-environmental conditions were favourable.

DECLARATION

I hereby declare that the research reported in this thesis is my own work except where due acknowledgement is made to other authors.

None of the work reported here has previously been submitted in whole, or in part, in candidature for any degree either at the De Montfort University, or at any other university.

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1: INTRODUCTION

1.1 THE BRITISH AMPHIBIANS

The term amphibian is derived from the Greek (Amphi-bios) meaning double life, and this reflects the split of most amphibian life cycles (including all British species) into aquatic larval and terrestrial adult stages. Amphibians are an integral part of many ecosystems often constituting the highest fraction of vertebrate biomass (Burton and Likens 1975). There are 6 amphibian species which are commonly accepted as being native to the U.K, three urodeles: *Triturus helveticus, T. vulgaris, T. cristatus* and three anurans: *Rana temporaria*, *Bufo bufo* and *B. calamita*. The two toad species (*B. bufo* and *B. calamita*) are the most terrestrial spending the majority of their adult life on land only returning to the water to breed. This thesis concentrates on the common toad (*B. bufo*). To avoid confusion all other toad species will be referred to by their full common or Latin names.

The Common Toad (*B. bufo*)

1.1.1 The Adult Phase

The genus *Bufo* has an almost world wide distribution, individual species may range over wide areas, including *B. bufo* which is Europe's most widespread amphibian.

B. bufo have been recorded from 65° north throughout mainland Europe to parts of Asia, Japan and NW Africa (Morrison 1994).

The most distinguishable morphological feature of bufonids are raised glands, most noticeable on the dorsal surface. *B. bufo* has prominent glands and also a series of dark markings in rows down the dorsal surface. The base colour of *B. bufo* is very variable from yellow-brown to olive green, often with well developed and highly visible paratoid glands behind the eyes (Morrison 1994). The sexes of *B. bufo* can be easily distinguished in the breeding season by the presence of dark-brown horny nuptial pads on the inner three digits of the male (Smith 1951). Bufonids are unable to leap to escape predation and have distasteful or sometimes toxic cutaneous secretions as an anti-predator strategy (Toft 1980).

B. bufo may be aged successfully by counting the year rings in phalanges (Hemelaar 1981) and using this technique in the Netherlands, Hemelaar (1983) showed adults can reach between five and six years. Gittins *et al* (1982) found male *B. bufo* in a Welsh population reached sexual maturity at two years and females at three years after metamorphosis. Reading (1988) showed that age to sexual maturity varies between sites even within a small geographical region. Reading (1988) found that the earliest age to sexual maturity for male *B. bufo* varied from two to three years at two different Dorset sites. Age to sexual maturity may vary within a single site during different years as shown by Hemelaars' long term study of a population in the Netherlands (Hemelaar 1983). First time breeding males were four years in the 1980 breeding season (Hemelaar 1983) whereas in the previous five breeding seasons (1975-1979) males had sexually matured at three years (Hemelaar 1981).

After the first breeding season females in an English population of *B. bufo* showed no correlation between age and size reflecting the large energetic cost of gamete production (e.g. Reading 1988). The cost of egg production results in females maturing later than males and thus, on reaching the breeding site, females are 1-3 years older and larger than the males (e.g. Jorgensen 1986). The later sexual maturation of female *B. bufo* and the shorter time spent at the breeding site contributes to the skewed

sex ratios observed by many workers (e.g. Gittins *et al* 1980, Hoglund and Robertson 1987). The skewed sex ratio has also been observed in other bufonids including *B. americanus* (Oldham 1966) and *B. calamita* (Banks and Beebee 1986a).

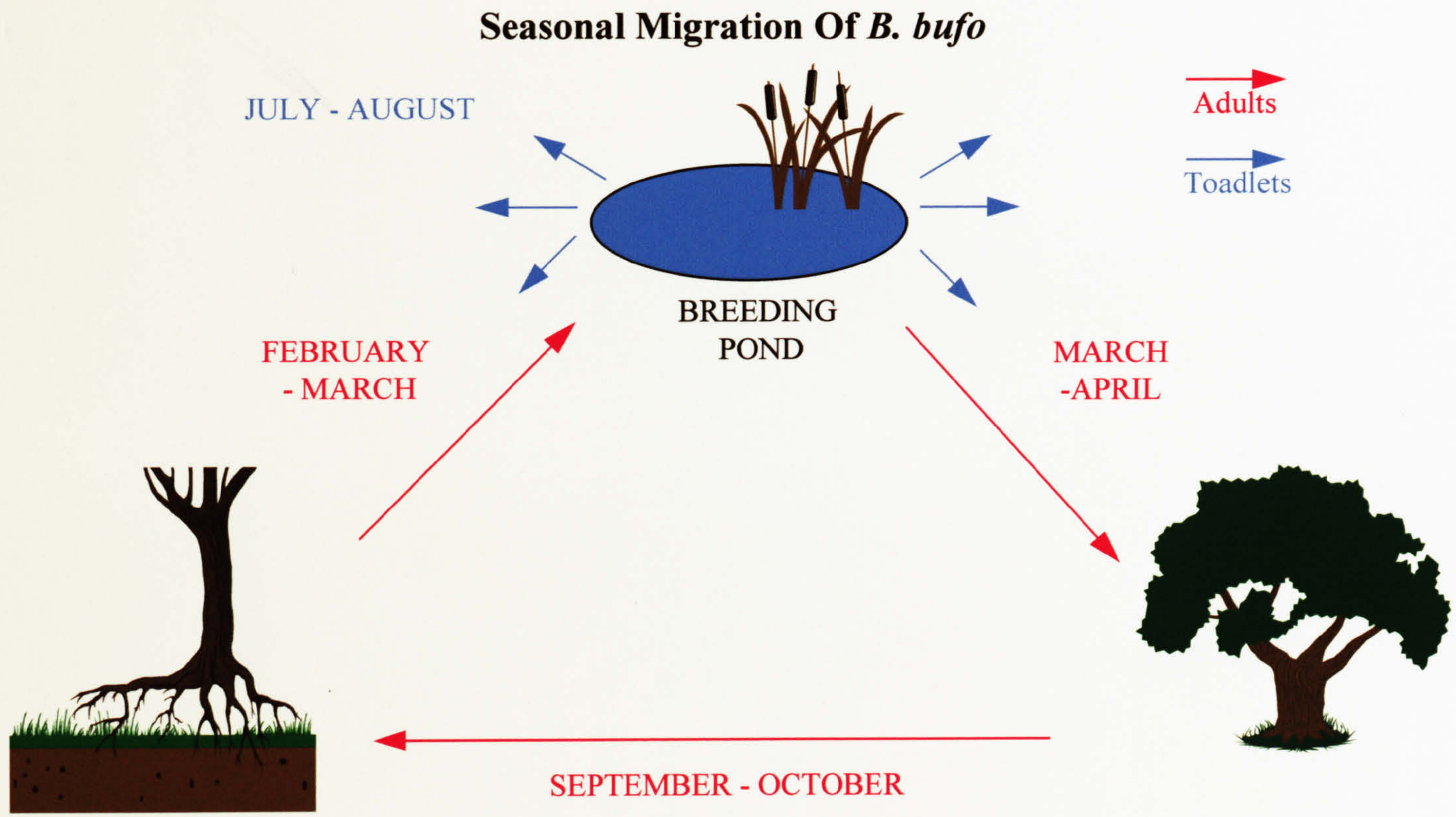
Most of the species within the *Bufo* genus occupy a similar ecological niche. They are usually nocturnal/crepuscular, terrestrial outside the breeding season, wide-ranging with distinctive migratory patterns. The seasonal migrations of *B. bufo* are summarised in Figure 1.1 (Pg 4). *B. bufo* is terrestrial outside the breeding season preferring scrub, woodland and rough grassland to more open habitats (e.g. Beebee 1981). A fuller discussion of *B. bufo* habitat preference is given in chapters 3 and 8 of this thesis.

Most adult bufonids including *B. bufo* are considered to be generalist sit-and-wait predators, feeding on a wide range of ground-dwelling arthropods particularly Coleoptera and Formicidae (e.g. Clarke 1974, Toft and Duellman 1979). Larsen (1984) gives an excellent review of feeding behaviour, physiology and ecology of *B. bufo*. A further discussion and review of the subject are given in Chapters 4 and 5 of this thesis.

1.1.2 Breeding Migration

B. bufo is an explosive breeder with intense scramble competition for mates (e.g. Wells 1977, Hoglund and Robertson 1988). The timing of the *B. bufo* breeding season will be controlled by prevailing climatic conditions (Frazer 1989, Wisniewski *et al* 1981) in central Britain *B. bufo* usually spawns in late March or April (e.g. Swan 1986) but in Southern England *B. bufo* may spawn in February or earlier (e.g. Reading and Clarke 1983). The main trigger for the breeding migration of *B. bufo,* as with many other amphibians, is temperature (Oldham 1966, Gittins *et al* 1980, Gittins 1983a). Threshold temperatures of 0.5°C to 8°C have been quoted (e.g. Frazer 1966, Gittins *et al* 1980, Wisniewski *et al* 1981, Reading and Clarke 1983, Sinsch 1988) with initiation temperatures varying with population (Oldham 1966) and method of recording (Sinsch 1988).

Figure 1.1



Adult *B. bufo* leave over-wintering sites in early spring and migrate "en masse" to the breeding site (e.g. Gittins *et al* 1980). After breeding, adults migrate to a summer home range to feed. In mid-summer the newly metamorphosed toadlets emerge from the breeding pond. In autumn the adults migrate to an over-wintering site which is often between the breeding pond and the summer home range. A detailed review of *B. bufo* migration patterns is given in Sinsch 1988

Environmental stimuli such as day length and relative humidity also play important roles in the timing of the breeding migration of *B. bufo* (Gittins *et al* 1980, Wisniewski *et al* 1981). If the ambient temperature is below threshold levels no *B. bufo* movement occurs even if relative humidity is 100% (Swan 1986). Once environmental conditions allow, *B. bufo* take a direct line to the breeding site reaching speeds between 100 and 250 m per night (Moore 1954, Gittins *et al* 1980) though migratory speed is influenced by vegetation (Van Gelder *et al* 1986a). Amphibians orientate towards the breeding site using a mixture of olfactory and visual clues (Oldham 1966, Sinsch 1987a, 1991) with up to 96% of surviving adult *B. bufo* returning to their original natal pond (Reading *et al* 1991). Breeding sites are found on a wide range of substrates and geology, varying from granite quarries to Chalk hill dewponds (Beebee 1977a) but breeding ponds are usually deep and permanent (Cooke 1975, Mathias 1975, Swan and Oldham 1989).

At low male densities *B. bufo* are more likely to call to attract females but at higher densities proportionally more males are silent but active, apparently searching out the females (Hoglund and Robertson 1988). Males will pair with the first unattached female they find but many females will be paired before they reach the spawning site (Davies and Halliday 1978, Reading and Clarke 1983). Male *B. bufo* will often try to pair with single or attached males, prompting the male release call (Davies and Halliday 1977, 1978, Hoglund and Robertson 1987). After breeding, adult *B. bufo* may enter a state of inactivity until temperatures reach a critical level (10 - 12°C) when they begin their active feeding (Jorgensen 1986, Sinsch 1988).

The number of eggs laid is usually about 600-4000, with larger females producing higher numbers of eggs (Swan 1986, Reading 1986). The relationship between female body size and fecundity varies with the individual, population and year (Gittins *et al* 1980, Reading 1986).

1.1.3 Development Of Tadpoles And Juveniles

Common toad eggs are able to develop in water of pH 4.2 -8.2 and an equally variable ion content (Cooke 1975). Eggs can hatch within 4 days at laboratory temperatures of

18-20°C (Goater 1994). At ambient temperatures normal in Spring, *B. bufo* take less than 10 days to hatch but cling to the remaining embryonic jelly for up to a further 10 days before becoming free-swimming larvae (Smith 1951, Morrison 1994). *B. bufo* tadpoles are uniformly black and in Britain can be confused with the very similar *B. calamita* tadpoles. Larvae of British bufonids can be identified by either measurement of mouth width (e.g. Smith 1951, Beebee 1983a) or by electrophoretic fingerprinting (Beebee 1990).

Most amphibian larvae, including *B. bufo,* are suspension filter feeders; transporting water through structures specialised to entrap particles (Jorgensen 1966, Diaz-Paniagua 1985, 1989). Amphibian larvae are important in the nutrient cycling of a pond; their grazing can affect the structure of the periphyton community and metamorphosis results in a net export of nitrogen from the aquatic ecosystem (Dickman 1968). *B. bufo* tadpoles are phytophagous consuming filamentous and single celled algae with some diatoms and protozoa (Savage 1952, Scorgie 1980, Diaz-Paniagua 1989).

Food quality and quantity have been shown to influence larval growth rate, size at metamorphosis and length of larval period in a range of anurans (e.g. Alford and Harris 1988, Dash and Mahapatro 1990, Johnson 1991, Kupferberg *et al* 1994). Both Savage (1952) and Diaz-Paniagua (1989) noted the comparatively short guts of *B. bufo* suggesting a short passage time and therefore probable low assimilation of food. Beebee (1983b) found that *B. bufo* fed *ad libitum* grew 1.5mm per week and Scorgie (1980) showed an increase in mass of up to 10.6mg per day of *B. bufo* in the field.

Both intra and interspecific density dependent competition have been demonstrated in a range of anuran larval species (e.g. Wilbur 1977, Dash and Hota 1980, Griffiths 1991). Abiotic factors, such as pond duration (Tejedo and Recques 1994a, 1994b) and biotic factors, such as parasitism (Goater 1994) may interact with competitor density to determine metamorphic success.

The results of Griffiths (1991) using replicated ponds, suggest that competition between *R. temporaria* and *B. calamita* is mediated by food depletion (resource competition) and by labile growth inhibitors (interference competition). These labile growth inhibitors were later identified as *Prototheca richardsi* (Beebee 1991, Wong and Beebee 1994) a unicellular, non-pigmented contramensal alga which requires passage through tadpole gut to sustain its replication and cell cycle. *P. richardsi* mediated inhibition of *B. calamita* larvae by both *R. temporaria* and *B. bufo* has been demonstrated in the laboratory and in replicated pond studies (Banks and Beebee 1987, Beebee 1991, Griffiths *et al* 1991). The importance of *P. richardsi* in mediation of interspecific competition at field densities remains contentious (Petranka 1995).

Anuran larval survival is also dependent on the level and type of predation. British bufonid larvae are distasteful and are thus avoided by most vertebrate predators though there are records of predation by *T. cristatus* (e.g. Cooke 1974). Denton and Beebee (1997) found that the presence of predatory fish increased the percentage survival of *B. calamita* in experimental ponds due to preferential predation of potential invertebrate predators. Large invertebrate larvae such as *Dytiscus* species can prey upon anuran larvae by piercing the tadpoles and sucking out the insides thus avoiding skin toxins (Swan 1986, Pearman 1995*)*. *T. helveticus* will also take *B. bufo* tadpoles at first but will soon learn to avoid them (Reading 1990). Despite the protection of skin toxins, *B. bufo* larval and metamorph survivorship is very low, with survival from eggs to first year juveniles of only 0.8 % (Swan and Oldham 1989).

B. bufo metamorphose during late June/early July in Leicestershire (Swan 1986). The timing of anuran metamorphosis is related to many factors including growth rate, food availability and temperature (e.g.Wilbur and Collins 1973, Smith-Gill and Berven 1979, Beck 1997). For example pond desiccation can result in early metamorphosis and smaller size of metamorphs (Audo *et al* 1995). Adult size at breeding is partly determined by that at emergence (Swan 1986). *B. bufo* from emergence until their first over-wintering period are referred to as metamorphs throughout the rest of this thesis. Metamorphs disperse at approximately right angles from the natal pond with both

movement and feeding continuing until the end of their first summer when they can reach up to 1Km from the pond (Oldham 1985, Oldham and Swan 1991).

1.2 DECLINING AMPHIBIAN POPULATIONS

1.2.1 Global Declines

Throughout the world serious declines in different species of frogs, toads and salamanders have been reported (e.g. Hayes and Jennings 1986, Phillips 1990, Bradford *et al* 1994). Certain taxa and geographic areas seem more affected: In North America most reports have concerned the genera *Rana* and *Bufo* (e.g. Hayes and Jennings 1986, Crump *et al* 1992). Bufonidae has more species endangered than any other family (e.g. Johnson 1992) and ranid frogs have almost disappeared from southern California (e.g. Blaustein and Wake 1990, Drost and Fellers 1996). In general, upland and more northern species are most severely affected (e.g. Wake 1991, Carey 1993).

Amphibian populations undergo cyclical fluctuations in population numbers (e.g. Pechmann *et al* 1991). These are in part a result of environmentally influenced reproductive cycles and demographic structure which affect population dynamics (e.g. Halley *et al* 1996, Guha *et al* 1980). Superimposed on these endogenous population dynamics are broad climatic patterns which may cause range expansion and contraction. There may also be synergism between these natural fluctuations and added environmental stresses producing greater impacts of environmental contaminants at range edges (e.g. Pechmann and Wilbur 1994).

In an attempt to separate natural fluctuations from real declines, and to establish any global trends, the National Research Council (USA) sponsored a workshop on global amphibian declines in February 1990. The overall conclusion of the workshop was that the declines seemed to be general and real but some regions and many taxa remain unaffected (Wake 1991).

The question remained; were these declines just part of the global biodiversity crises described by many workers (e.g. Wilson and Peter 1988) or are amphibians bioindicators of a particular global problem? Three factors suggest the latter may be the case. Firstly many of the declines and extinctions have occurred in protected, undeveloped and apparently

pristine environments (e.g. Bradford *et al* 1994, Drost and Fellers 1996). Secondly many reports suggest that amphibians may be particularly sensitive to anthropogenic impacts due to their ecology, physiology and behaviour (e.g. Duellman and Trueb 1986). Thirdly declines in amphibians seem to be relatively recent and global, occurring simultaneously in many diverse species whilst other taxa in the same areas remain unaffected (Wake 1991). The diversity of species and countries in which population declines have been observed suggests a global phenomenon such as ozone depletion and the concomitant increased exposure to UV-B may be the cause (e.g. Blaustein *et al* 1994).

Hard evidence for a global declines of amphibians, greater than those of other taxa are difficult to find as natural fluctuations make definition of a decline difficult to formulate and test (Pechmann and Wilbur 1994). Reasons for local declines and extinctions are easier to establish. Declines of the Houston toad (*B. houstensis*) and the Wyoming toad (*B. hemiophrys baxteri*) followed loss of terrestrial and breeding habitats (Johnson 1992). Studies on introductions of predatory fish have shown significant effects on amphibian populations ranging from behavioural changes to local extinctions (e.g. Kats *et al* 1988, Semlitsch 1993, Bronmark and Edenhamn 1994). Introduction of the bullfrog *R. catesbeiana* has also been a cause of declines of several species including *R. boylii* (Kupferberg 1997) and Blanchards cricket frog *Acris crepitans blanchardi* (Lannoo *et al* 1994).

Acidification of breeding sites has long been reported as a cause of deformities and deaths of amphibia (e.g. Beebee 1986). The growth rates of frogs and toads may be affected by even short term acid conditions (e.g. Freda and Dunson 1986). Pesticides, herbicides and other pollutants may all play a role in local and possibly global extinctions of amphibia. Pollutants may act as direct toxins: Hall and Henry (1992) reviewed the toxic effect of more than 54 chemicals on amphibians. Environmental contaminants may also impair the immune system and increase susceptibility to disease (e.g. Nyman 1986, Bradford 1991) or increase the likelihood of predation (Wasserberg and Sperry 1977). Even increases in road traffic density seem to be important for amphibian declines (Fahrig *et al* 1995). There may be synergistic effects between any of these local effects and global phenomena associated with climate change and ozone destruction.

1.2.2 Declines In Britain

British species are not immune to this seemingly world wide trend with declines in both rare and commonly occurring species. Both *B. calamita* and *T. cristatus* have been afforded protection under the Wildlife and Countryside Act (1981). The many studies on the status of *B. calamita* confirm its' threatened status (Beebee 1977b, Banks and Beebee 1986a and 1986b, Sinsch 1991). In an NCC report (Cooke and Scorgie 1983) on endangered amphibians and reptiles, *B. calamita* had disappeared from Suffolk, Hampshire and the North Wales coast. The main reasons for the severe declines of this bufonid include the reclamation of heathland, an increase in coastal tourism, encroachment by *B. bufo,* acidification of breeding sites and large scale afforestation especially in North Wales (Beebee *et al* 1990). Declines in the number of breeding sites and suitable terrestrial habitats are also responsible for the decline in populations of *T. cristatus* (Cooke and Scorgie 1983).

The other British amphibians are also suffering population losses. A number of studies have found declines in both *R. temporaria* and *B. bufo* numbers in the United Kingdom since the 1940's (Cooke 1972, Cooke and Ferguson 1974, Beebee 1977b). In 1990 the NCC undertook an enquiry into the status of the "commoner" British amphibians, including *B. bufo,* on the British mainland (Hilton-Brown and Oldham 1991). Though *B. bufo* was still widespread and common, numbers had declined in many central and southern areas compared with populations recorded in 1982 (Cooke and Scorgie 1983).

B. bufo declines, like those of *T. cristatus*, were thought to be mainly due to loss of habitat in rural areas. Changes in agricultural practices have influenced amphibian populations particularly in areas of extensive blanket arable crops (Cooke and Ferguson 1974). Though habitat loss is probably still the major cause of amphibian loss in Britain, concern is growing over the effects of pollution. For example anthropogenic acidification of surface waters can be a problem for British amphibians. Tadpoles of *R. temporaria* at low pH showed reduction in growth rates and increased percentage deformity (Cummins 1989). Beebee *et al* (1990) gave evidence that

anthropogenic acidification of breeding sites had contributed to the reduction in the distribution of the *B. calamita* in the 1980's.

1.3 RATIONALE FOR THE PRESENT STUDY

B. bufo is an omnivorous widespread small vertebrate and has an important niche in the ecological energetics of the British countryside. Declines in populations of British amphibians, including *B. bufo*, are well documented (Cooke 1972; Beebee 1977b, 1981, Cooke and Ferguson 1974, Cooke 1975, Cooke and Scorgie 1983, Swan and Oldham 1989, Grayson *et al* 1991, Oldham and Swan 1991). Management strategies and conservation proposals based upon sound ecological knowledge are required to combat this trend.

Studies on *B. bufo* have tended to concentrate on the aquatic larval stage (e.g. Scorgie 1980, Banks and Beebee 1987, Beebee 1990) or on the adults during the breeding season when *B. bufo* migrate "en masse" to the breeding site. (e.g. Gittins *et al* 1980, Gittins 1983a, 1983b, Cooke 1975, Davies and Halliday 1977, Banks and Laverick 1986, Sinsch 1988, Reading *et al* 1991). The terrestrial niche: habitat selection, feeding behaviour, time budgets and basic physiological constraints of the life of *B. bufo* outside the breeding season have been less well studied and are the subject of the current study.

1.4 AIMS

This thesis aims to determine the abiotic and biotic factors which influence the habitat selection of *B. bufo* in an agricultural environment, emphasising the importance of the terrestrial habitat.

1.5 APPROACH AND THESIS STRUCTURE

Since *B. bufo* are cryptically coloured nocturnal/crepuscular vertebrates there are problems in simply locating them. An appropriate method is radio-tracking. However tracking of *B. bufo* presents problems of transmitter attachment, therefore the second chapter of this thesis is dedicated to experiments into the best method of tag attachment. The development of the radio-tracking system is described in chapter 3. Radio-tracking can provide data on where *B. bufo* spend their time, further study is

required to elucidate the reasons for habitat preference. Abiotic and biotic correlates of habitat selection were therefore monitored in conjunction with the radio-tracking. *B. bufo* are ectothermic, arthropodivores and their habitat preference is likely to be influenced by microclimate and prey availability. These were measured using a combination of monitoring and experimentation.

Prey availability and diet were monitored by a range of sampling methods which are described in chapters 4 and 5. Microclimate of selected refugia was monitored and compared with non-refugia microclimate and this is discussed in chapter 6. The hypotheses developed from these chapters were tested by relating population size of 30 field populations to habitat use in chapter 7. Chapter 8 describes the interactions between all these factors and discusses the results and implications of the work described in the previous 7 chapters.

2: TAG ATTACHMENT

2.1 INTRODUCTION

Transmitters can be attached externally to a range of animal species using collars, harnesses, bands or clips. Externally attached transmitters can cause a variety of problems ranging from an increased risk of predation (Garrott *et al* 1985) to hair or feather loss and skin irritation (Bartholomew 1967, Hessler *et al* 1970, Corner and Pearson 1972, Greenwood and Sargeant 1973, Kenward 1982, Hines and Zwickel 1985). Transmitter mass to body mass ratios become increasingly important with smaller animals. Large transmitters have impaired both movement (Banks *et al* 1975) and digging ability (Corner and Pearson 1972), as well as reducing survival in small mammals (Brooks and Banks 1971).

External attachment of transmitters to amphibians presents particular problems due to the elastic and delicate nature of amphibian skin. Amphibian skin is tough but once punctured is easily torn making clip attachment impossible. Problems of external attachment are increased by the regular sloughing of amphibian skin. Despite the problems, amphibians have been tracked using externally attached transmitters (Van Nuland and Claus 1981, Van Gelder *et al* 1986a, Sinsch 1987a,1988,1992).

Externally attached transmitters cannot be used for long term studies as the ability of the amphibian to bury in refugia would be compromised. To overcome the problems of external attachment, radio transmitters can be fitted internally, either by forced ingestion or by surgical implantation. Transmitters have been surgically implanted into a range of snake species (Madsen 1984, Kroll *et al* 1972, Lutterschmidt and Reinert 1990, Reading and Davies 1996) and into several amphibian species: *B. boreas* (Carey 1978), *R. catesbeiana* (Stinner *et al* 1994), *B. calamita* (Sinsch 1988, 1992, Denton and Beebee 1993) and *Buergeria buergeri* (Fukuyama *et al* 1988). Ingested transmitters were used by Oldham and Swan (1992) for *R. temporaria* and *B. bufo*. Ingestion of dummy transmitters was found to have no effect upon the feeding of either of the two species tested.

Both surgical implantation and ingestion of transmitters may cause a change in field activity. Surgically implanted transmitters have reduced the activity of small mammals (Hamley and

Falls 1975, Leutze 1980) whilst northern water snakes (*Nerodia a. sipedon)* showed reduced activity and aberrant post-ingestion behaviour when transmitters were ingested (Lutterschmidt and Reinert 1990). In home range and habitat preference studies it is essential that a tagged animal behaves in the same way as an untagged animal (Kenward 1982). It is therefore important to test the effects of transmitter attachment on any study species before conducting a tracking study. The overall aim of this chapter was to assess the affect of ingested and surgically implanted transmitters on *B. bufo.*

2.2 THE PRELIMINARY INVESTIGATION 1993

2.2.1 Approach

To determine the most suitable method for tagging of *B. bufo,* laboratory experiments were conducted in August 1993. The methods of attachment chosen for the investigation were surgical implantation and forced ingestion. The objective of the 1993 experiment was to assess if indicator variables could be used to assess the effects of transmitter attachment on *B. bufo.* Since stressed *B. bufo* loose mass and show reduced feeding (Larsen 1990) mass change and feeding rate were chosen as the indicator variables.

2.2.2 Methods

The investigation began on 16th August and was terminated on the 22nd September. Transmitter life under field conditions was usually between four and ten weeks, the length of the investigation (five weeks) reflected the expected transmitter life. Dummy transmitters were used of the same shape and size (mean mass 2.67 +/-0.057g) as Biotrack S2 transmitters, which were to be used in field studies (chapter 3). The dummy transmitters were made from fuse wire coated in an inert silicone.

Fifteen *B. bufo* obtained from a relatively dry field pen were kept in water overnight in an attempt to obtain re-hydration of the animals. After hydration, *B. bufo* were separated randomly into three groups with five animals in each. Ideally *B. bufo* used in the experiment would be chosen from a single sex population of even size, however, due to difficulty in obtaining animals in 1993, animals were of varied sizes and both male and female. Initial variations in mass and sex specific differences will increase the standard errors of mass and feeding data. This reduces the level of control in the experiment and

may mask any treatment specific differences in feeding or mass. However, using both sexes of varied size ensured a cross section of adult animals were sampled.

B. bufo were kept individually in plastic aquaria (22.5 x 32.5 x 20.0 cm) with a soil and litter substrate and a glass lid. A petri dish of water ensured high humidity and prevented desiccation, and the weekly mass of *B. bufo* was recorded. *B. bufo* were presented a recorded number of crickets every day (0.54 g +/-0.04 g) always in excess of the number of crickets eaten. *B. bufo* were monitored for one week prior to the insertion and implantation of transmitters and for one week after tags were removed. Five animals were given ingested transmitters on 24th August following the method used by Oldham and Swan (1992). On 22nd September transmitters were removed by forcing the animals to regurgitate them by a gentle pressure on the belly. Any tags regurgitated during the course of the investigation were reinserted, and the number of regurgitations recorded.

B. bufo were surgically implanted on the 24th and 27th August using the surgical procedure described by Sinsch (1988) for *B. calamita.* All surgical procedures were performed under Home Office Licence. Before surgery, *B. bufo* were anaesthetised using 0.2% MS-222 (0.2g/l) until the leg reflex was unresponsive. After cleaning the abdomen with antiseptic, a 1 cm incision was made in the abdominal flank. Skin and musculature were cut, enabling the tag to be placed inside the abdominal cavity. The wounds were then stitched with 3-4 "*Surucor*" absorbable sutures (Suru Group, Bombay, India) and the animals allowed to recover under supervision. On 20th and 22nd September tags were removed from animals using the same procedure used for insertion, however the removal incision was made on the opposite side of the abdomen to the original incision. The five remaining animals were used as the controls. These animals were weighed weekly and fed daily as described above but did not undergo any implantation or ingestion procedures.

2.2.3 Statistical Analysis

Statistics were conducted using SPSS and MINITAB statistical packages. Two repeated measures, one-way analysis of variance (ANOVAs) were carried out, one on the total number of crickets per week with treatment as the between subject variable and week number as the within subject variable. The second ANOVA used mass of *B. bufo* as

dependent variable, with treatment as between subject variable and week as within subjects variable. The mass of the animal at the end of week one (prior to tag insertion/implantation) was used as a covariant in the second analyses in an attempt to control for initial variation in size of animals.

2.2.4 Results

During the investigation period a fatality of one *B. bufo* with an ingested transmitter occurred. This individual was heavily parasitised on *post mortem* by the lung nematode (*Rhabdias bufonis*). All animals with ingested transmitters regurgitated their transmitters at least once during the investigation period, with one animal regurgitating its tag seven times.

(i) Feeding Rate

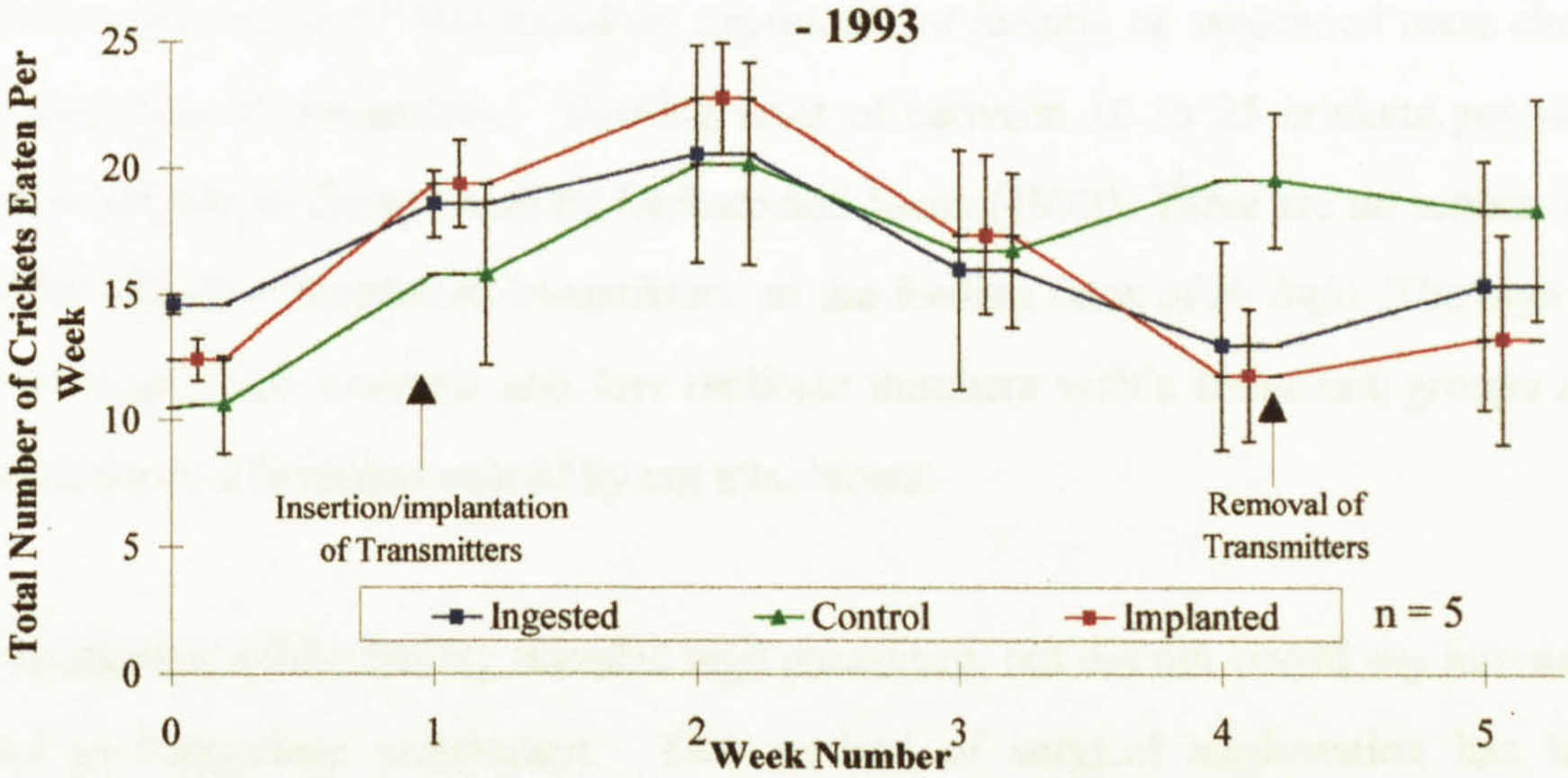
The feeding rate was calculated as the number of crickets eaten per week. The error bars on Figure 2.1 demonstrate the large within treatment variation in numbers of crickets eaten. The feeding rates of animals did not differ significantly between the three treatments ($F_{2,12}$=0.06, P>0.05) and treatment and time did not interact significantly ($F_{6,36}$=1.07, P>0.05). Feeding did differ significantly with time ($F_{3,36}$=2.95, P<0.05). Numbers of crickets eaten ranged from a minimum of seven per week in week four to a maximum of 25 per week in week two.

(ii) Mass

The mass of *B. bufo* during the investigation are shown on Figure 2.2. There was no significant difference in the mass of *B. bufo* either with treatment or with time ($F_{2,11}$=0.19, P>0.05; $F_{3,36}$=2.69, P>0.05 respectively) and the two factors did not interact significantly ($F_{6,36}$=0.30, P>0.05). As expected the covariant (initial mass) showed a significant correlation with mass during the investigation ($T_{1,11}$ = 9.981, P<0.001).

Figure 2.1

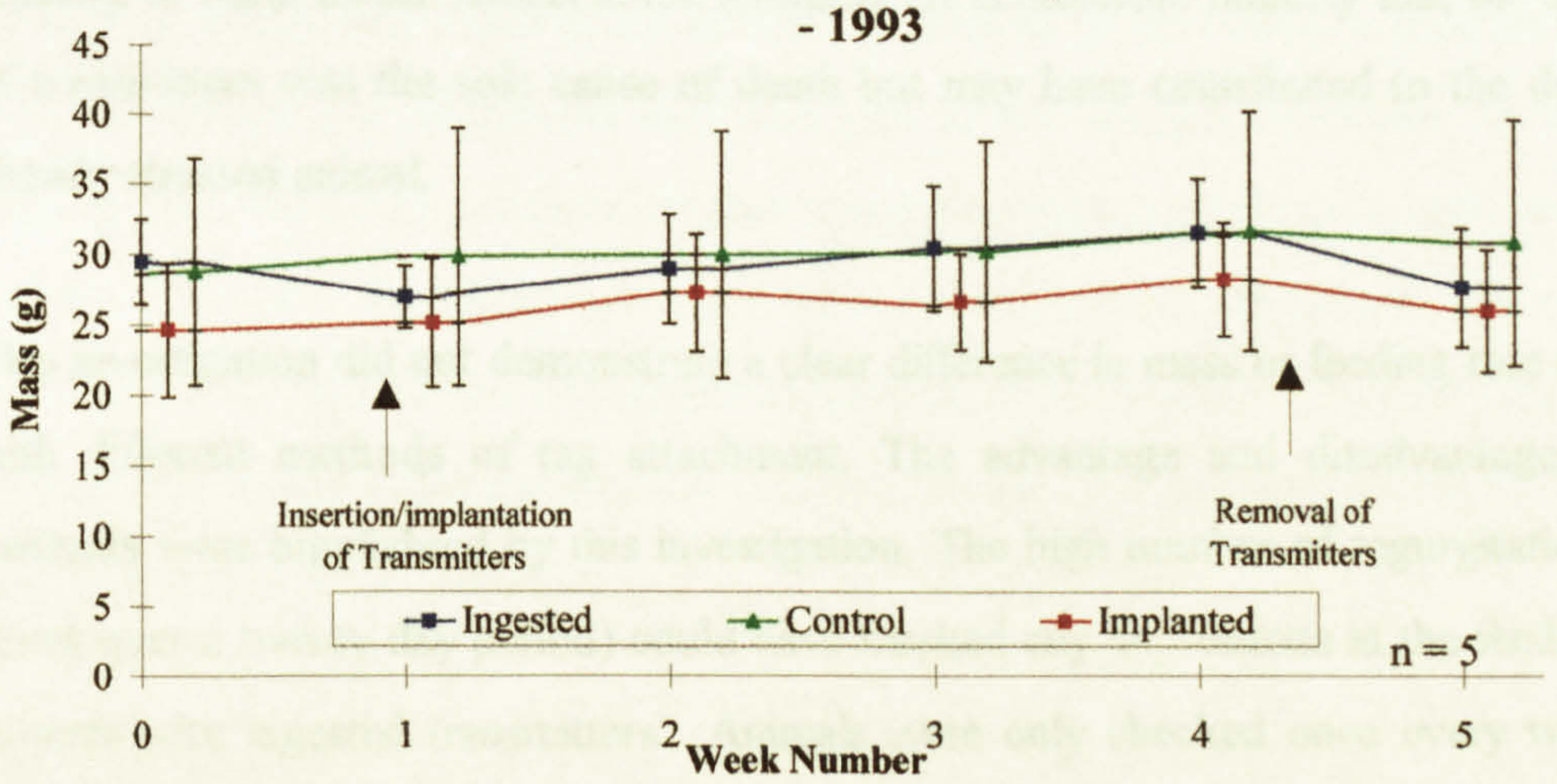
Feeding Of *B. bufo* With Ingested And Implanted Transmitters - 1993



Error Bars show +/- 1 standard error of the mean. There were no significant differences in the number of crickets eaten by toads either between treatments or with time (P>0.05). Number of crickets eaten was recorded daily and then summed to provide weekly totals for each individual toad. Data points are staggered for clarity. n = number of animals per treatment.

Figure 2.2

Mass Of *B. bufo* With Ingested And Implanted Transmitters - 1993



Error Bars show +/- 1 standard error of the mean. There were no significant differences in the mass of toads either between treatments or with time (P>0.05). Mass of toads within each treatment were recorded in the same 24 hour period, however data points are staggered for clarity. n = number of animals per treatment.

2.2.5 Discussion

Neither method of tag attachment significantly affected mass or feeding in *B. bufo*. Oldham and Swan (1992) found no depression of feeding or associated mass changes due to ingestion of transmitters. Feeding rates of between 10 to 25 crickets per week were comparable with those found by Oldham and Swan (1992). There are no reports of studies of the effects of implanted transmitters on the feeding rates of *B. bufo*. The high variance, wide confidence intervals and low replicate numbers within treatment groups may have masked any differences caused by tag attachment.

Post mortem of the fatality revealed high parasitism, but did not reveal any internal damage due to transmitter attachment. This method of surgical implantation has been used successfully by Sinsch (1988) in *B. calamita* and by Denton and Beebee (1993, 1994) for both *B. calamita* and *B. bufo*. None of these studies reported any wound infection in the field, but no controlled laboratory studies were undertaken.

The fatality with the ingested tag showed no distension of the stomach wall, nor any bruising or burst blood vessels in the stomach. It is therefore unlikely that the attachment of transmitters was the sole cause of death but may have contributed to the death of an already stressed animal.

This investigation did not demonstrate a clear difference in mass or feeding rate of *B. bufo* with different methods of tag attachment. The advantage and disadvantages of both methods were highlighted by this investigation. The high number of regurgitations (up to seven over a twenty day period) could have masked any depressions in the feeding rate of animals with ingested transmitters. Animals were only checked once every twenty four hours, and the exact time of the regurgitation was unknown, therefore animals may have fed between regurgitation and re-insertion of the tags. Regurgitation of transmitters may also cause problems and inaccuracies in long term (longer than six weeks) field based studies resulting in loss of both animals and data.

Surgically implanted transmitters are not regurgitated and would therefore not be subject to these problems. Surgical implantation however, is complicated to perform and cannot be

performed without a licence. Both surgical implantation and ingestion of transmitters may cause reduction in field activity. The activity of *B. bufo* in the field with ingested radio transmitters is discussed in chapter 3.

On the basis of these results both surgical implantation and ingestion are suitable methods of transmitter attachment for short term studies of *B. bufo*. The results from this investigation must be treated with caution due to the high initial variation in mass and the low numbers of replicates.

2.3 SEX SPECIFIC DIFFERENCES IN RESPONSE TO TAGGING 1994

2.3.1 Introduction

It is possible that the large within treatment variations in the 1993 experiments may have been due to sex specific differences. *B. bufo* show sex specific differences in size and age to sexual maturity (Hemelaar 1983, 1985). Females can take up to 2-3 years longer to mature sexually, due to the energetic cost of egg production (Hemelaar 1983). The stress of egg production may result in sex specific differences in the response to internal tagging. The male/female size dichotomy may also lead to sex specific differences in response to tagging. Smaller males will have a higher transmitter mass to body mass ratio compared to the larger females. The 1994 experiment aimed to discover if a sex specific difference in the response of *B. bufo* to the implantation/ingestion of transmitters could be demonstrated. Response to tagging was measured in terms of changes in mass and feeding rate of tagged and untagged (control) animals.

2.3.2. Methods

30 *B. bufo*, 15 males and 15 females taken from a wet field pen, were left to re-hydrate in water for two hours and then weighed on 31st July 1994. They were then randomly assigned to three groups of ten animals with five males and five females in each category: control, implanted and ingested. Animals were kept for one week before implantation and insertion of transmitters or use as control animals. *B. bufo* were kept under the same conditions as in the 1993 experiments. Implantation and ingestion followed 1993 procedures and animals used as controls were not subjected to any ingestion or

implantation procedures. Males were implanted on the 7th August females on the 8th August. *B. bufo* were presented crickets daily in excess, and number of crickets eaten was recorded daily, mass was recorded weekly. Crickets were of even size from the same instar and weighed before *B. bufo* were fed to ensure even food quantities were provided. *B. bufo* were checked twice daily to reduce times between regurgitation and reinsertion.

2.3.3 Statistical Analysis

Statistical analysis was conducted using SPSS and MINITAB packages. Data were checked for any significant deviations from normality before statistical analysis was conducted. A repeated measures, two-way ANOVA was carried out on the total number of crickets per week, with treatment and sex as the between subject variables and week number as the within subject variable. A second repeated measures ANOVA used *B. bufo* mass as the dependent variable, with the same independent and within subject variables. The mass at the end of week one (prior to tag insertion/implantation) was used as a covariant in the second analyses. When overall differences were demonstrated by the ANOVAs, a Tukey test was conducted to determine which factors and weeks were significant (For details see Fowler and Cohen 1990).

2.3.4 Results

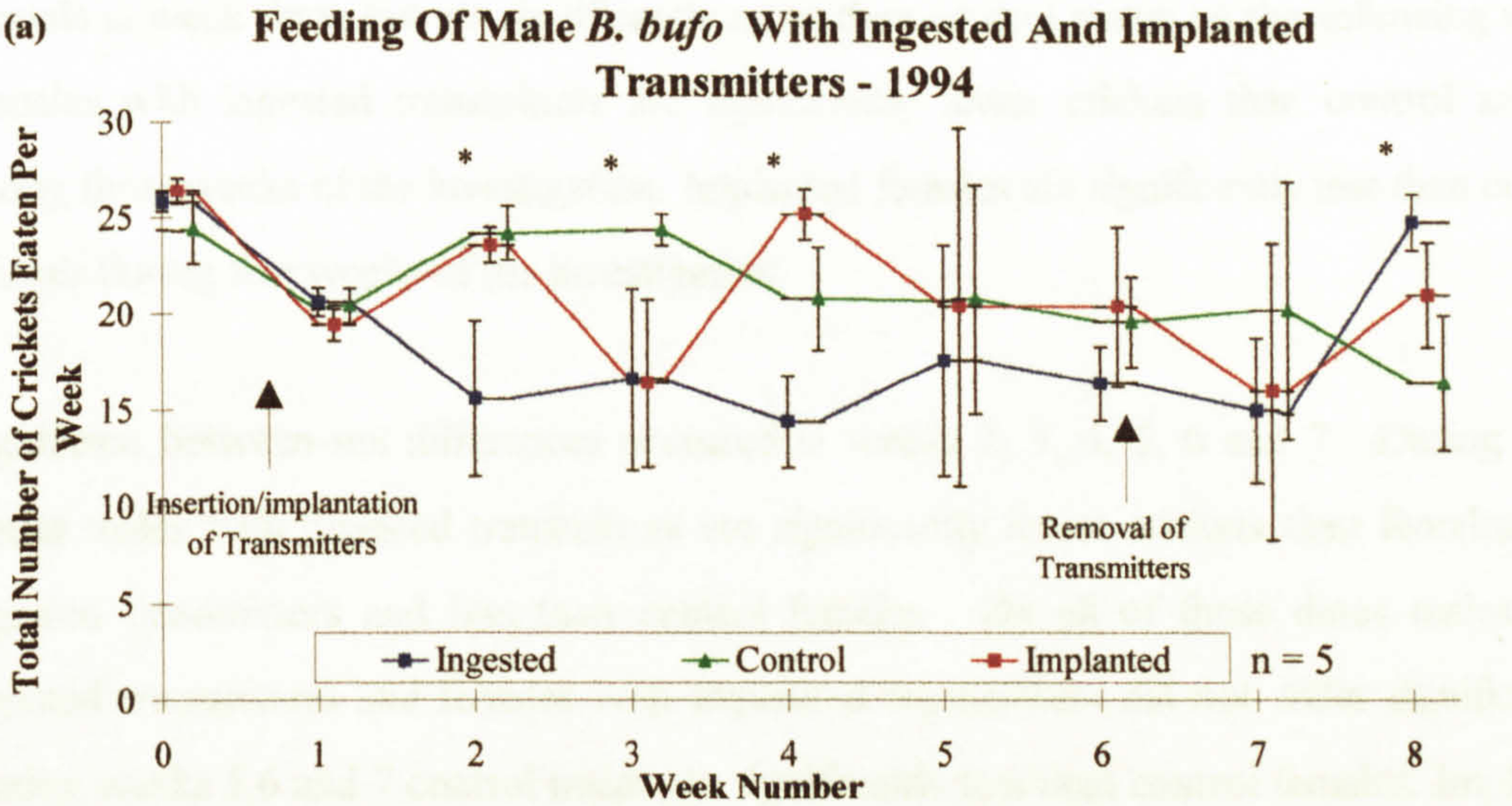
(i) Feeding

Feeding of male and female *B. bufo* are shown in Figures 2.3a and 2.3b respectively. Variation in feeding of male *B. bufo* was initially small but increased after treatment in animals with ingested transmitters. Feeding rates of *B. bufo* differed significantly both between sexes ($F_{1,23}$=5.17, P<0.05) and with time ($F_{6,138}$=2.49, P<0.05). Though there was no significant difference between treatments alone ($F_{2,23}$=1.58, P>0.05) there was a significant interaction between sex and treatment ($F_{2,23}$=3.72, P<0.05). There were also significant interactions between sex and time ($F_{6,138}$ = 4.18, P<0.01) and between sex, treatment and time ($F_{12,138}$ = 2.52, P<0.01).

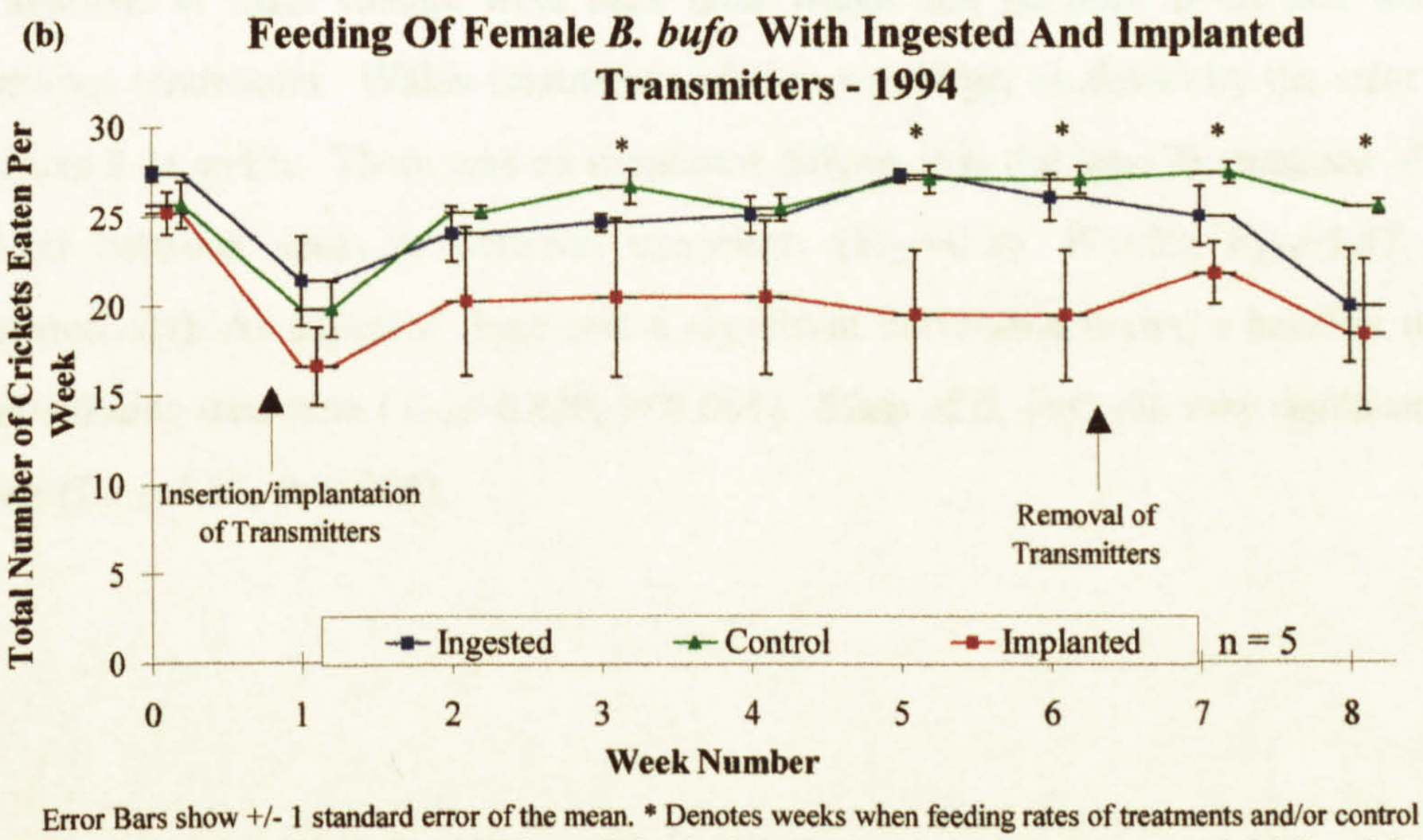
The significant within-sex, between-treatment differences are shown by * on graphs 2.3a and 2.3b.

Figure 2.3

Sex specific Differences In Feeding Of Tagged *B. bufo*



Error Bars show +/- 1 standard error of the mean. * Denotes weeks when feeding rates of treatments and/or control groups differed significantly (P<0.05;Tukey>tabulated value). Number of crickets eaten was recorded daily and then summed to provide weekly totals for each individual toad. Data points are staggered for clarity. n = number of animals per treatment



Error Bars show +/- 1 standard error of the mean. * Denotes weeks when feeding rates of treatments and/or control groups differed significantly (P<0.05;Tukey>tabulated value). Number of crickets eaten was recorded daily and then summed to provide weekly totals for each individual toad. Data points are staggered for clarity. n = number of animals per treatment

Males with ingested transmitters ate significantly less than control males during three weeks of the investigation. Males with implanted transmitters ate significantly less than control animals in week three but ate significantly more than control males on the following week. Females with ingested transmitters ate significantly fewer crickets than control animals during three weeks of the investigation. Implanted females ate significantly less than control animals during five weeks of the investigation.

Significant between-sex differences occurred in weeks 2, 3, 4, 5, 6 and 7. During these weeks males with ingested transmitters ate significantly fewer crickets than females with ingested transmitters and less than control females. On all of these dates males with ingested transmitters and females with implanted transmitters did not differ significantly. During weeks 5,6 and 7 control males ate significantly less than control females. Implanted males and females did not differ significantly in their feeding rates.

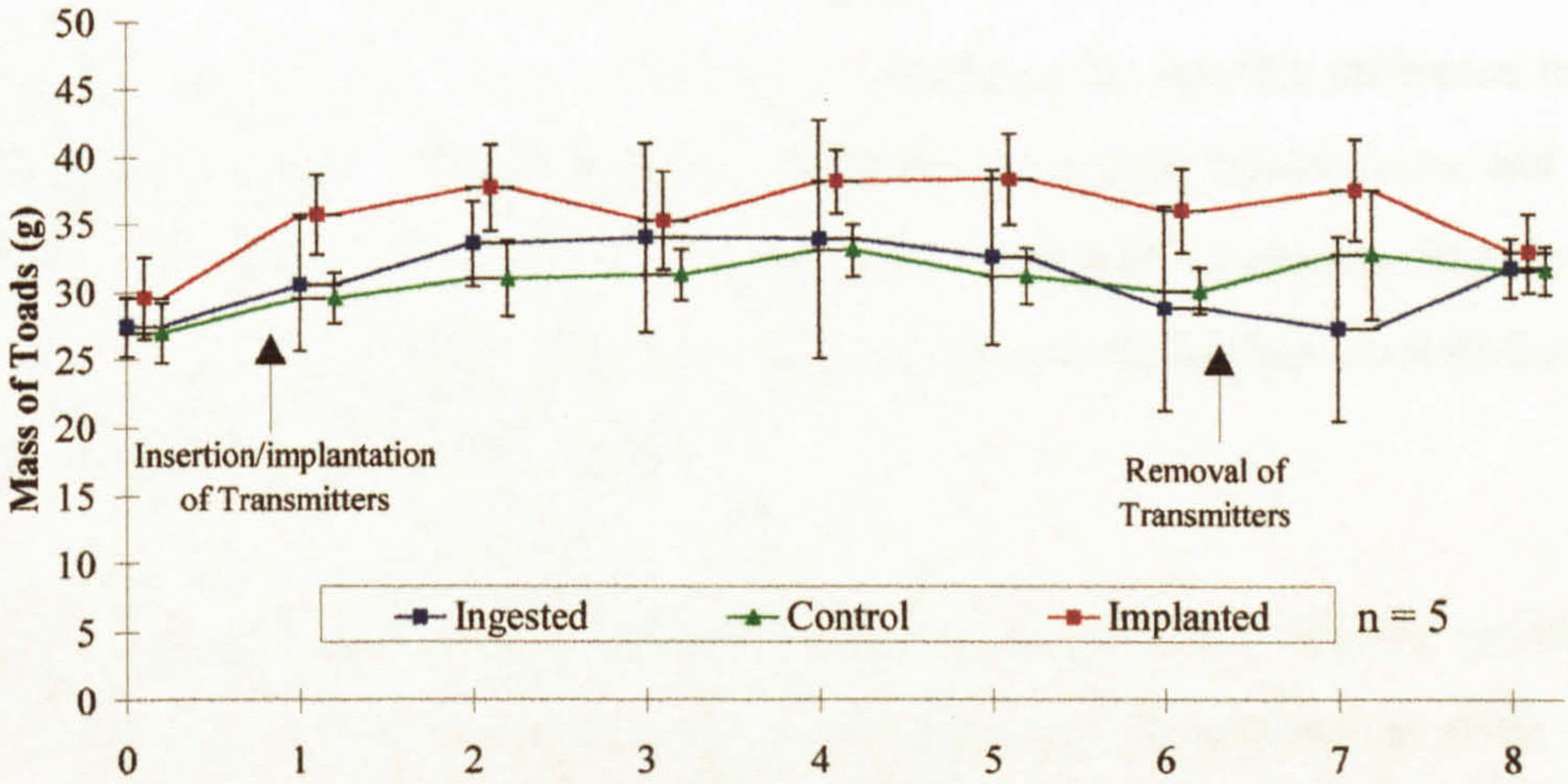
(ii) Mass

Mass changes of male and female *B. bufo* are shown in Figures 2.4a and 2.4b respectively. Variations in mass change were high both within and between sexes and within and between treatments. Within treatment variation was large, as shown by the error bars on figures 2.4a and b. There was no significant difference in the mass fluctuations of animals either between sexes or between treatments ($F_{1,23}$=0.39, P>0.05; $F_{2,23}$=1.47, P>0.05 respectively). As expected there was a significant correlation between baseline mass and mass during treatment ($T_{1,29}$=8.820, P<0.001). Mass of *B. bufo* did vary significantly with time ($F_{4,96}$=4.83, P<0.005).

Figure 2.4

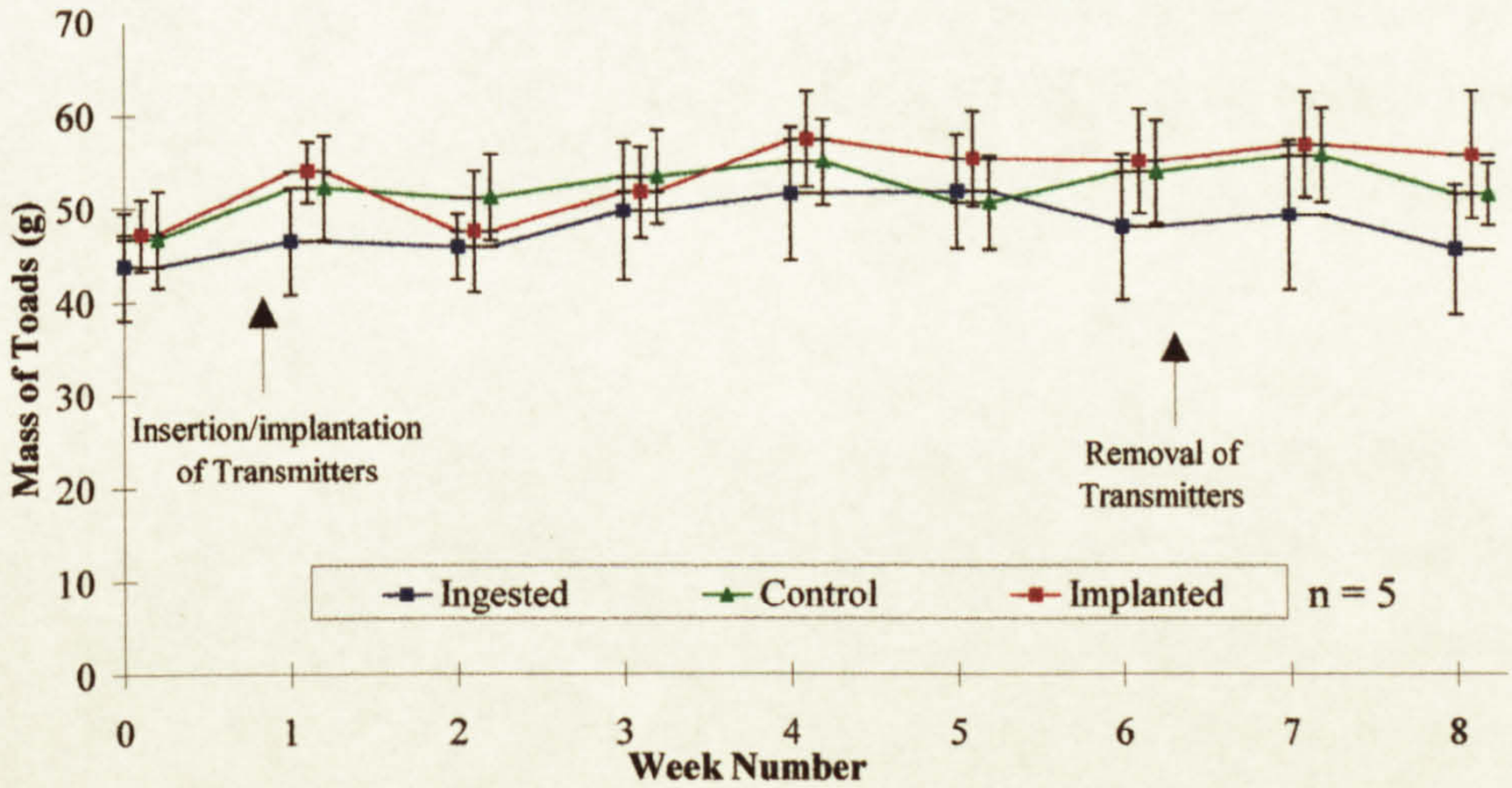
Sex Specific Differences In Mass Of Tagged *B. bufo*

(a) **Mass Of Male *B. bufo* With Ingested And Implanted Transmitters - 1994**



Error Bars show +/- 1 standard error of the mean. There were no significant differences in the mass of toads either between treatments or with time (P>0.05). Mass of toads within each treatment were recorded in the same 24 hour period, however data points are staggered for clarity. n = number of animals per treatment.

(b) **Mass Of Female *B. bufo* With Ingested And Implanted Transmitters - 1994**



Error Bars show +/- 1 standard error of the mean. There were no significant differences in the mass of toads either between treatments or with time (P>0.05). Mass of toads within each treatment were recorded in the same 24 hour period, however data points are staggered for clarity. n = number of animals per treatment.

2.3.5 Discussion

B. bufo males in the control group ate significantly less than their female counterparts. The higher nutritional requirements of females were probably due to a combination of their larger size and the high energetic cost of egg production. As well as innate sex-specific differences in nutritional demands there was a significant sex-specific difference in response to tagging methods. This resulted in a significant interaction between sex and treatment $F_{2,23}$=3.72, P<0.05). A significant depression in feeding of implanted females occurred throughout the experimental period (see figure 2.3b) whereas implanted males had only one week of significant feeding depression.

Males with ingested transmitters showed significant depression in feeding on three weeks out of eight whereas females showed significant depression in feeding only at the end of the experiment after transmitters had been removed. The effect of tagging by ingestion on male *B. bufo* is reversible as feeding rates recovered almost immediately after tags had been removed. In the only published study on the effects of ingested tagging on *B. bufo*, Oldham and Swan (1992) showed no significant difference between *B. bufo* with transmitters and control animals.

The reduction in feeding by females with implanted transmitters may be due to their internal morphology. The space in the body cavity of female *B. bufo* may be reduced by the growth of ovaries as the eggs develop. Pressure from implanted tags may cause stress and reduce female feeding rate.

Despite initially low variations in mass, post treatment variations were very high and no significant differences were found between sexes or between treatments. Graphs 2.4a and b appear to show a sex specific difference in the mass of males and females. This results from initial difference in mass and is therefore accounted for by the baseline mass covariant. This covariant is highly correlated to mass during the investigation ($T_{1,23}$=8.820, P<0.001). *B. bufo* increased in mass significantly over the two months of the investigation ($F_{4,96}$=4.83, P<0.005). The within treatment variance in mass may have resulted from variance in hydration both within treatment and with time.

The sex specific differences in response to tagging by ingestion were most likely due to the sex specific size dichotomy. Males in the wild (Gittins *et al* 1980, Hemelaar 1983) and in this investigation, were on average smaller than females, thus transmitter mass to body mass ratio was higher. The comparatively small reduction in feeding shown by implanted males suggests that the presence of an object in the stomach of the *B. bufo* is the major factor in the reduction in feeding. The lack of effect of transmitter ingestion on female *B. bufo* suggests that larger animals may not be affected by this method of tagging.

Small *B. bufo* were more likely to regurgitate transmitters, with three of the small *B. bufo* regurgitating their transmitters on more than three occasions. Only one large *B. bufo* regurgitated a tag and then only once. Oldham and Swan (1992) found that *B. bufo* regurgitated tags between 2-38 days after ingestion, with 69% regurgitating them within two weeks. They do not mention any size or sex specific differences in the rate of regurgitation or feeding rate of *B. bufo* in response to tagging.

Implanted transmitters have been widely used for radio-tracking and there is very little evidence of their affects on anurans. Sinsch (1988) used the implantation technique used in this study on *B. calamita* and he did not observe any wound infection. Denton and Beebee (1993 and 1994) tracked both *B. bufo* and *B. calamita* using the same implantation procedure. Neither set of researchers mention the effect of tagging on either species of anuran.

Carey (1978) implanted tags into *B. boreas* to monitor temperature. She measured the affect of tag implantation on body temperature by orally monitoring the temperature of animals without implanted transmitters. Carey found that implantation did not affect the body temperature of *B. boreas* as tagged and untagged body temperatures closely approximated one another. However she used only one animal and did not provide any statistical evidence for her statements. Van Nuland and Claus (1981) tracked anurans with external harnesses after observations of anuran behaviour in the laboratory. Van Nuland and Claus state that tagging did not inhibit mobility or feeding but again no empirical evidence is presented.

Extrapolation of laboratory results to field situations is always dubious however some conclusions can be drawn from this experiment. Tracking of small *B. bufo* with ingested transmitters would be likely to result in the loss of data due to tag regurgitation. Egg production/formation could be inhibited or reduced by the implantation of transmitters into female *B. bufo*. The significant reduction in feeding by males with ingested transmitters and in implanted females in the current study is also likely to alter behaviour and therefore affect field data collection.

2.4 DETERMINATION OF THE SIZE THRESHOLD FOR TAGGING BY INGESTION 1995

2.4.1 Introduction and Approach

In studies on the effects of tagging on the small animals much emphasis is placed upon the transmitter mass to body mass ratio (e.g. Greenwood and Sargeant 1973). The results of the 1994 investigation suggested that transmitters reduced the feeding in smaller *B. bufo* (usually male). The mass of transmitters is minimised as a matter of course, the only variable that could be manipulated was therefore the size of the study animal.

It was possible that the Biotrack S2 transmitters (to be used in field studies) may only be used for larger animals. The 1995 investigations aimed to determine whether there was a threshold limit in size of *B. bufo* suitable for tracking. To minimise the number of animals used only the method of forced ingestion was tested. This method was to be used for field radio-tracking (described in chapter 3) and was therefore of greatest relevance to the current study. The effects of tagging in terms of feeding and mass changes were compared for three size categories of *B. bufo* in laboratory studies during 1995.

2.4.2 Methods

Sixty male *B. bufo* were sorted by size into three groups: 20 large, 20 medium and 20 small. The baseline (initial) masses of the large group ranged from 25.3g to 30.4g, the medium group ranged from 20.6g to 25.1g and the small *B. bufo* from 18.3g to 20.3g. The snout-vent lengths of the large group ranged from 62.5mm to 65.1mm the medium group ranged from 58.6mm to 61.0mm and the small *B. bufo* from 53.0mm to 56.2mm.

Transmitters used for ingestion by the non-control animals were of fixed dimensions (19.04mm +/-0.59mm x 7.31mm +/-0.395mm) and mass (2.67 +/-0.05g). Daily feeding rates and weekly mass of *B. bufo* were recorded as in 1993. In an attempt to reduce variability in mass change due to moisture loss, the *B. bufo* were re-hydrated in water for two hours before weighing. This re-hydrated mass should reflect changes in tissue instead of a combination in moisture loss and tissue change. After a weeks acclimatisation to the experimental cages ten animals per size group were given ingested transmitters as described in Section 2.2.2. The remaining ten animals per size group were the controls and were not subjected to any of the tagging procedures. All *B. bufo* were presented a known mass of crickets (in excess) and were checked twice daily to reduce time between regurgitation and re-insertion of tags.

2.4.3 Statistical Analysis

Data were checked for any significant deviations from normality in MINITAB before statistical analysis was conducted on SPSS. A repeated measures, two-way ANOVA was carried out on the total number of crickets per week, with treatment and size as the between subject variables and week number as the within subject variable. A second repeated measures ANOVA used mass of *B. bufo* as the dependent variable, with the same independent and within subject variables. The mass at the end of week one (prior to tag insertion/implantation) was used as a covariant in the second analyses. When overall differences were demonstrated by the ANOVAs, a Tukey test was conducted to determine which factors and weeks were significant (For details see Fowler and Cohen 1990).

2.4.4 Results

(i) Feeding

The number of crickets eaten per week by large, medium and small sized *B. bufo* are shown in Figures 2.5a 2.6a and 2.7a respectively. The feeding of *B. bufo* varied significantly with time ($F_{3,72}$=8.09, P<0.001). The difference in feeding between large and small animals increased significantly with time ($F_{6,72}$=2.36, P=0.039).

There was a significant reduction in the feeding rates of *B. bufo* with ingested transmitters by comparison with control animals ($F_{1,24}$=24.16, P<0.001). Smaller *B. bufo* ate

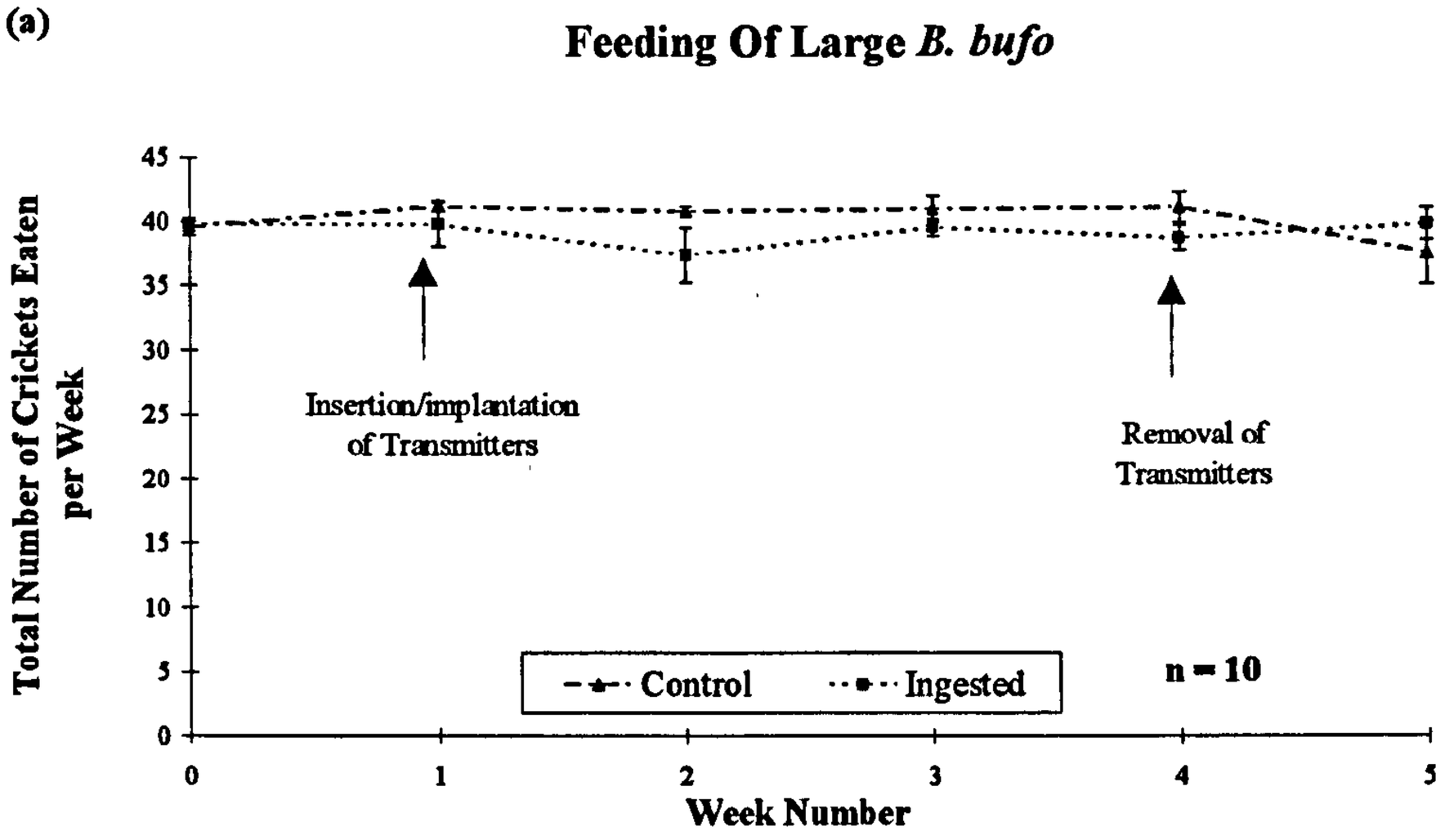
significantly less than larger animals ($F_{2,24}$=8.65, P<0.01). Both small and medium sized animals with ingested transmitters showed a significant reduction in feeding. The reduction in feeding rate in animals with ingested transmitters was greater in the small *B. bufo* compared with the medium sized animals ($F_{2,24}$=3.78, P=0.037). Large animals with ingested transmitters showed no reduction in feeding. The reduction in number of crickets eaten per week in treated animals compared with controls increased significantly with time ($F_{3,72}$=3.73, P=0.015). The variance in feeding rate of treated animals increases each week after treatment.

(ii) Mass

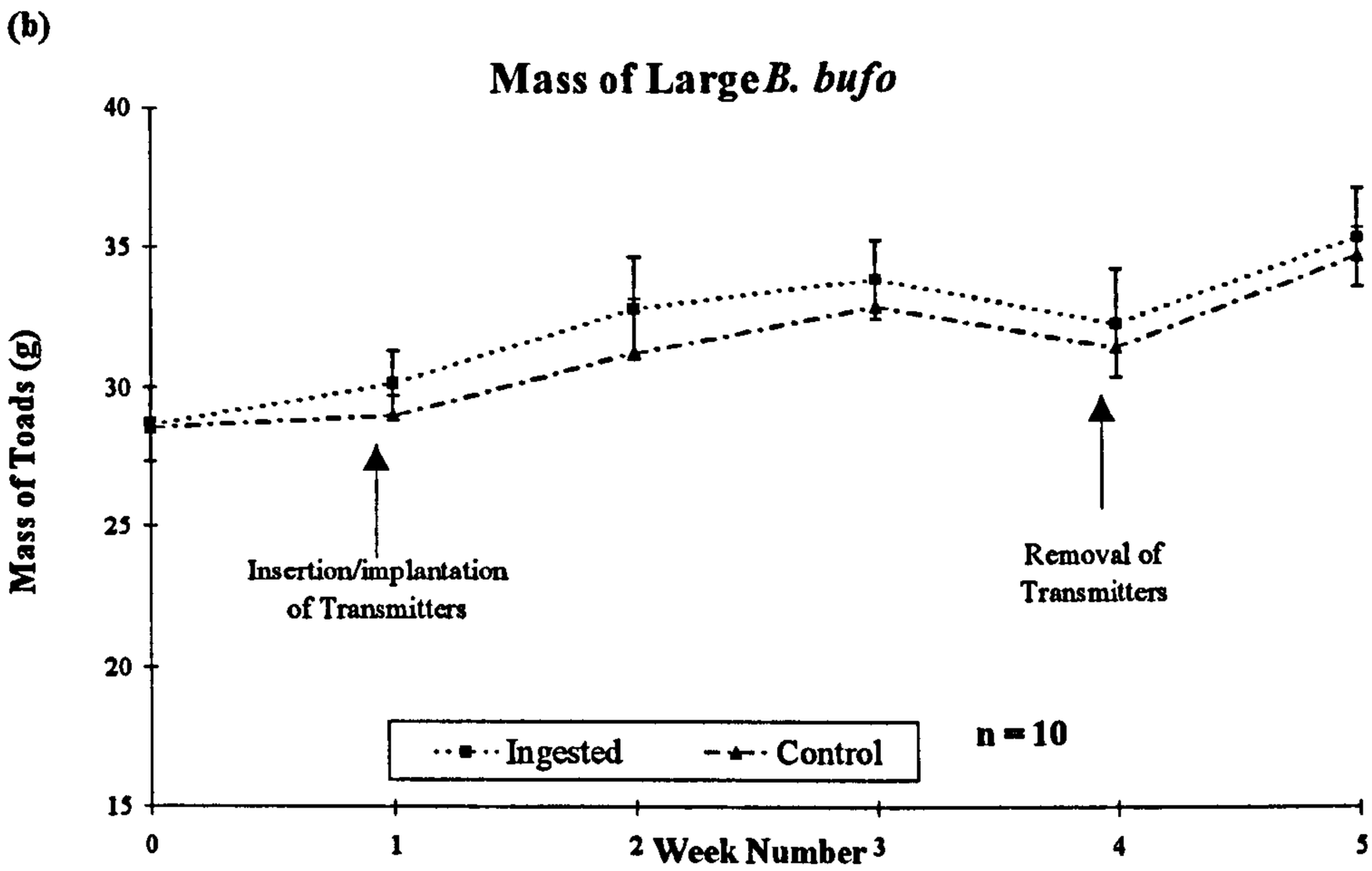
The masses of large, medium and small *B. bufo* are shown in Figures 2.5b, 2.6b and 2.7b respectively. As expected, there was a significant correlation between baseline mass and mass during the investigation ($T_{1,23}$=2.379, P<0.05). Masses of *B. bufo* varied significantly with time ($F_{4,96}$=19.74,P<0.001,) and there were significant interactions between size, treatment and time ($F_{8,96}$ = 2.41,P<0.05). The significant between-treatment differences are shown by * on graphs 2.5b, 2.6b and 2.7b. Large *B. bufo* with ingested transmitters showed no significant differences in mass compared with controls. Medium sized animals with tags had significantly lower mean mass than controls on week two of the investigation. Small animals with ingested transmitters had a significant reduction in mass from week three onwards, when compared with controls.

Figure 2.5

Response Of Large *B. bufo* To Tagging By Ingestion - 1995



Error bars show +/- 1 standard error of the mean. There were no significant differences in the numbers of crickets eaten by toads with ingested transmitters and controls (P>0.05).

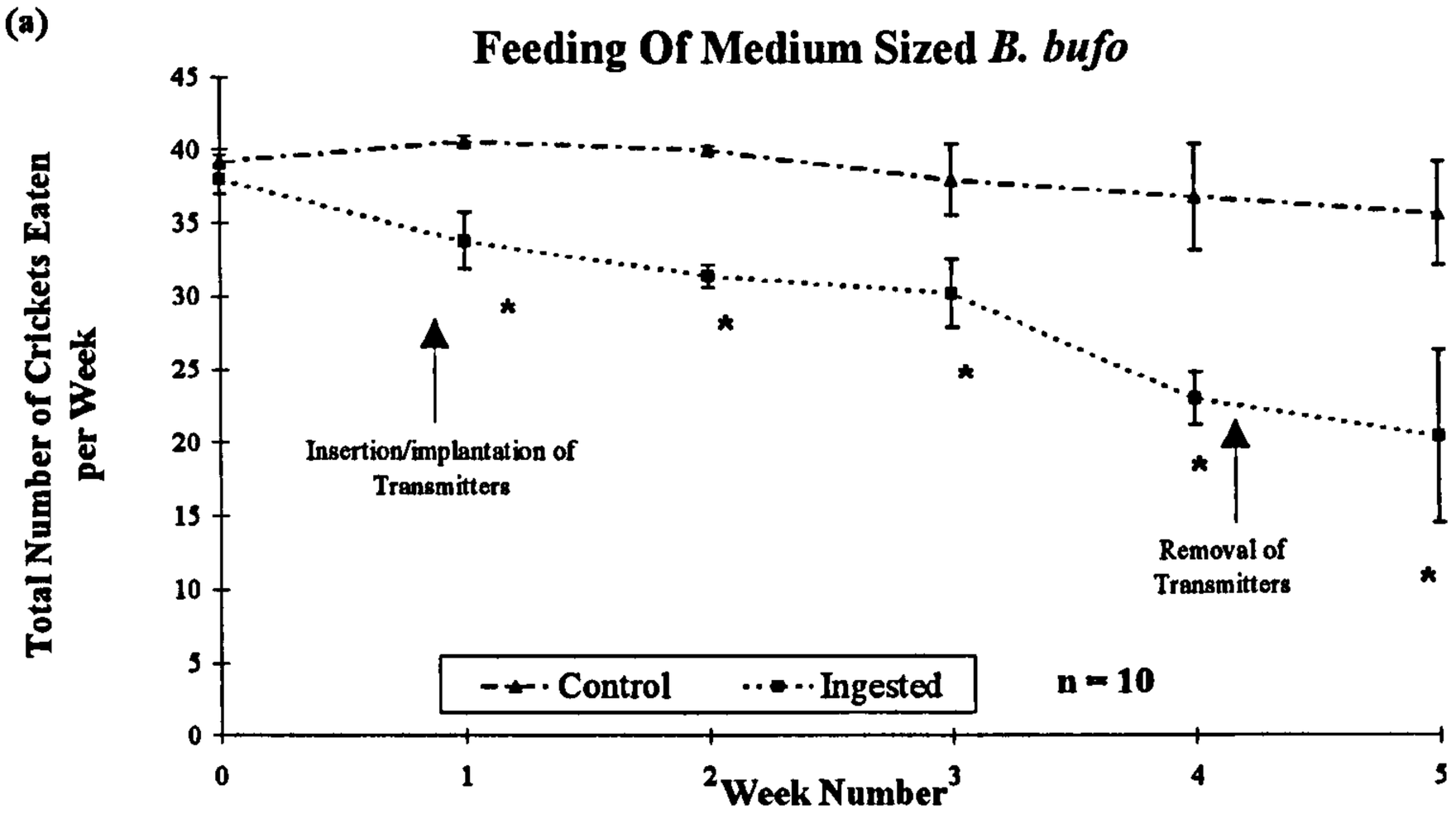


Error bars are +/- 1 standard error of the mean. There were no significant differences (P>0.05) in the masses of control and treated animals due to large within treatment variances.

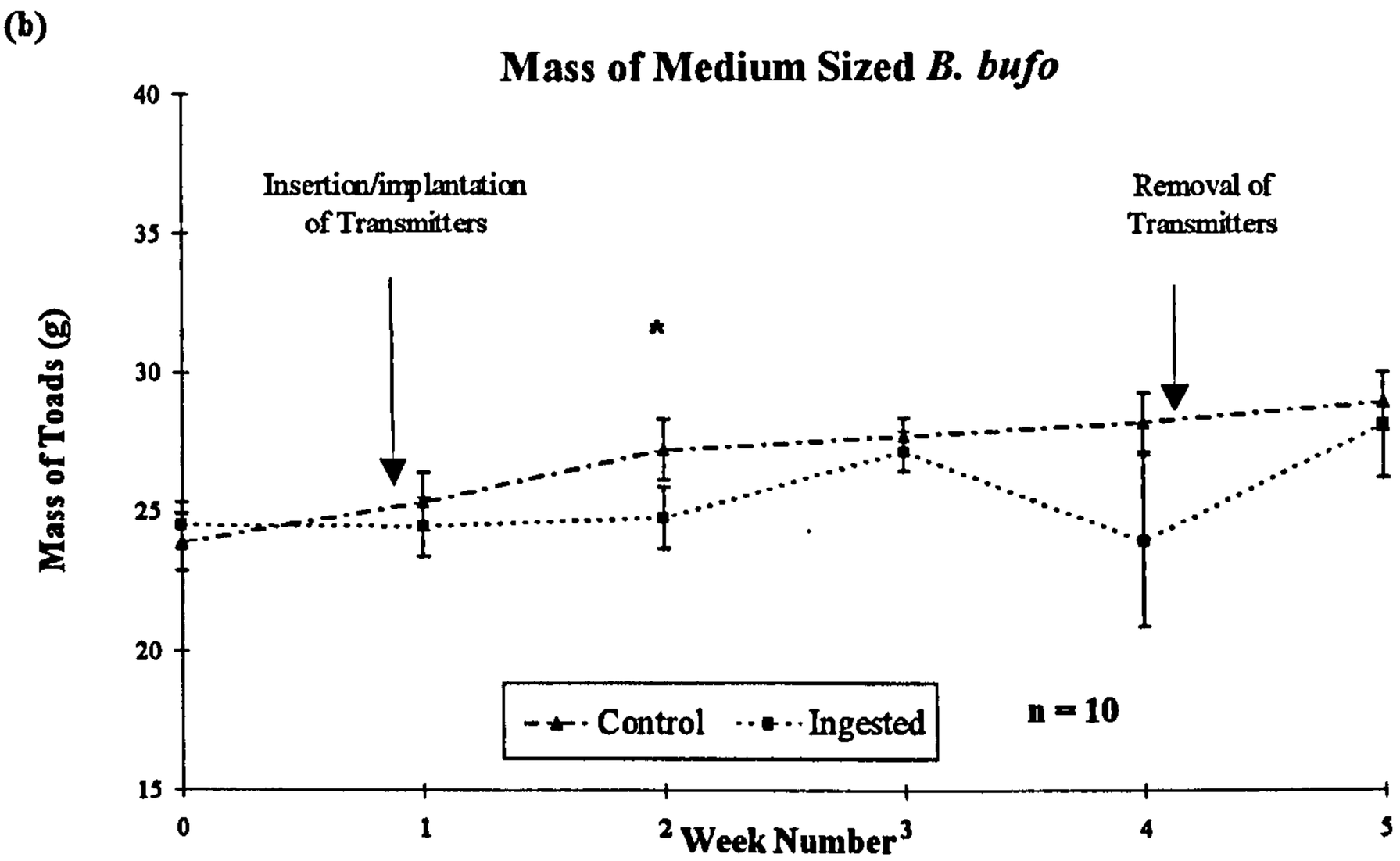
n = number of animals per treatment

Figure 2.6

Response Of Medium Sized *B. bufo* To Tagging By Ingestion - 1995



Error bars show +/- 1 standard error of the mean. * Denotes week when number of crickets eaten by toads with ingested transmitters was significantly less than numbers eaten by controls (P<0.05:Tukey T > tabulated value).



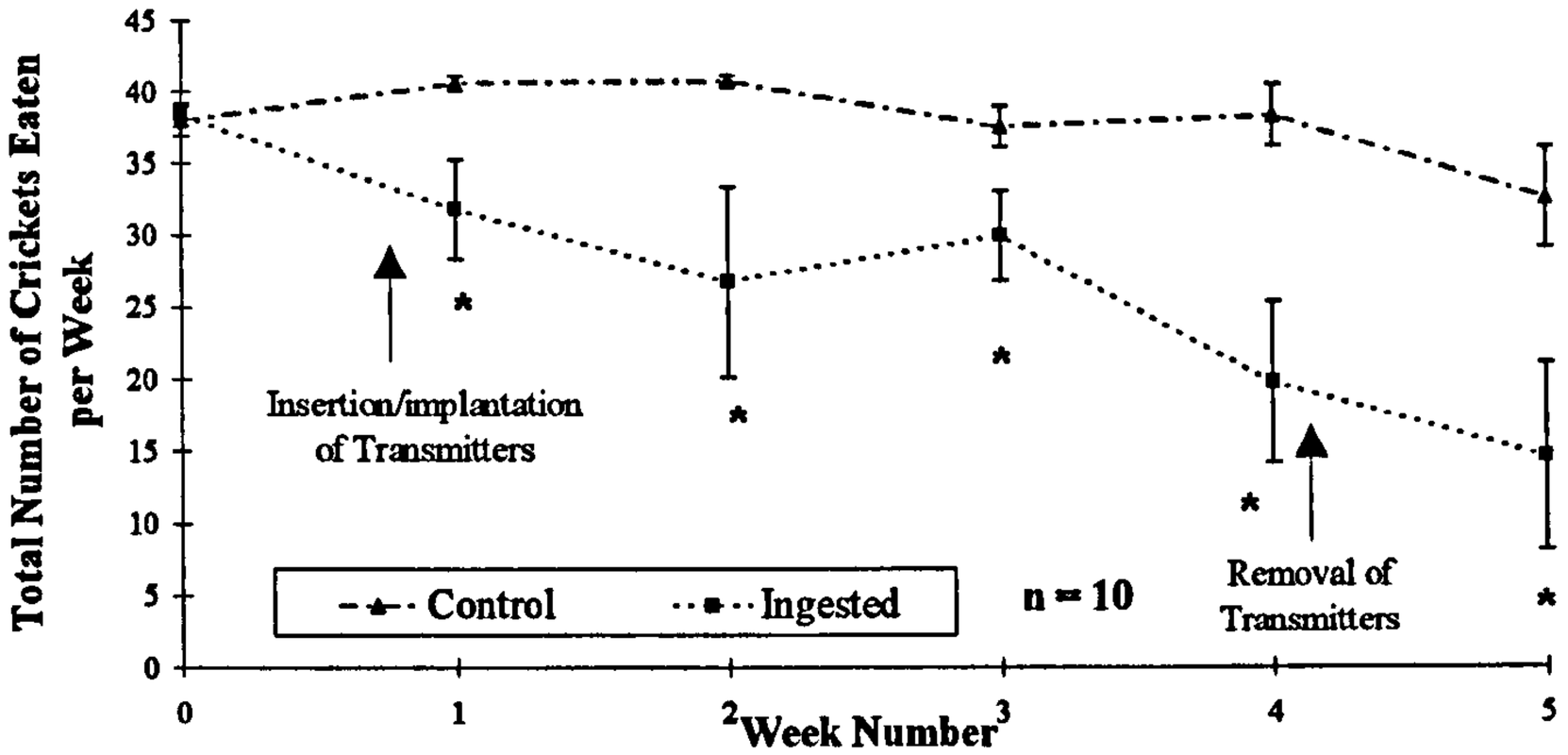
Error bars show +/- 1 standard error of the mean. * Denotes a week when mass of toads with ingested transmitters was significantly less than that of controls (P<0.05:Tukey T > tabulated value).

n = number of animals per treatment

Figure 2.7

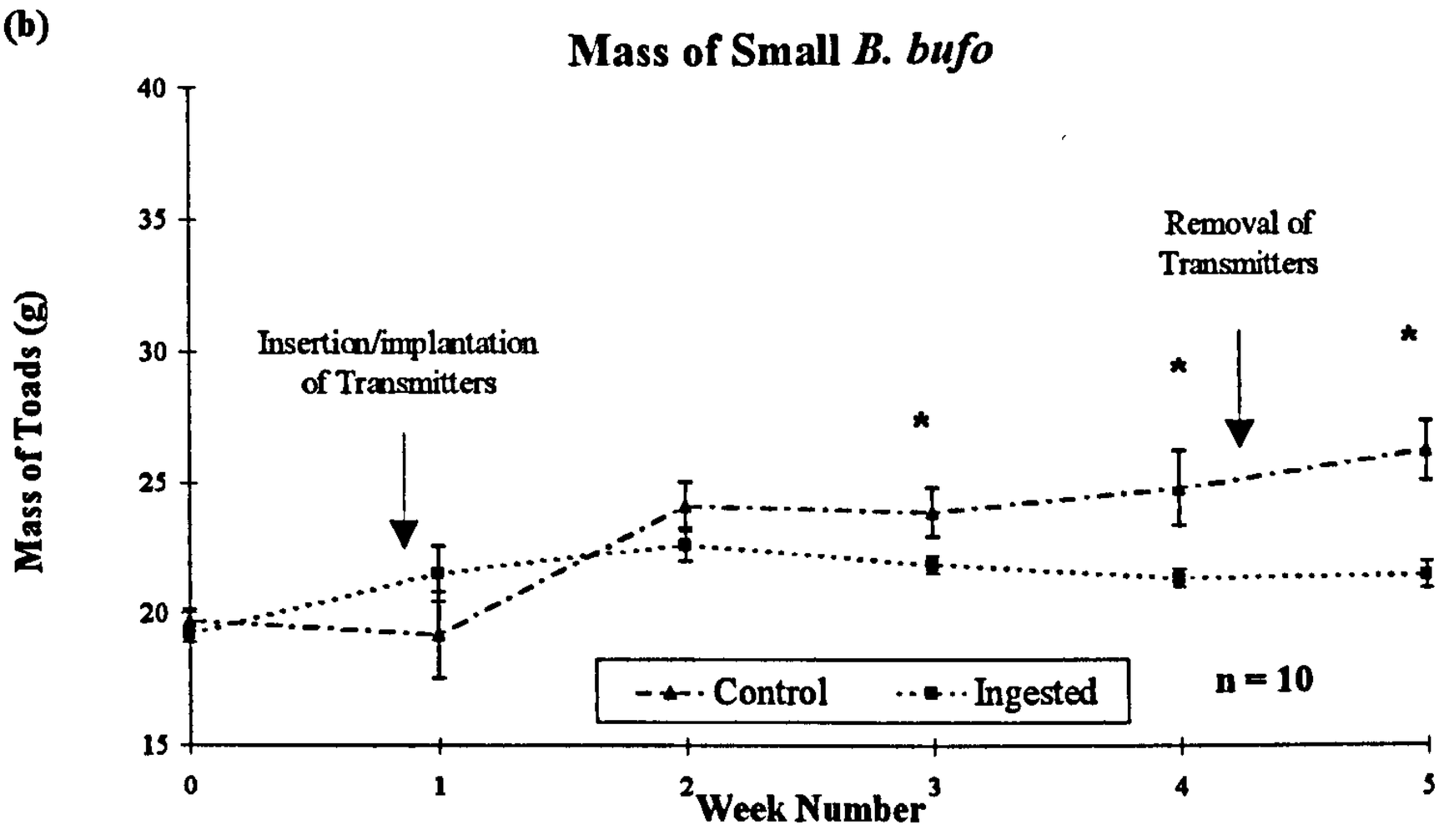
Response Of Small *B. bufo* To Tagging By Ingestion - 1995

(a)



Error bars show +/- 1 standard error of the mean. * Denotes week when number of crickets eaten by toads with ingested transmitters was significantly less than numbers eaten by controls (P<0.05:Tukey T > tabulated value).

(b)



Error bars show +/- 1 standard error of the mean. * Denotes week when mass of toads with ingested transmitters was significantly less than that of controls (P<0.05:Tukey T >tabulated value).

n = number of animals per treatment

2.4.5 Discussion

The larger *B. bufo* remained unaffected by tagging showing no significant reduction in feeding and no significant differences in mass compared with the controls. Tagging by ingestion did significantly affect the ability of small and medium sized *B. bufo* to feed ($F_{1,24}$=24.16, P<0.001). A significant reduction in mass of small *B. bufo* ($F_{8,96}$=2.41, P<0.05) resulted from the low nutritional intake due to the significantly reduced feeding. Medium sized animals with ingested transmitters had a mean mass gain, however masses were still significantly affected by tag ingestion compared with controls ($F_{8,96}$=2.41, P<0.05).

The lower limit of the large size group gives the mass for the threshold limit as 25g. Since the dummy transmitters had a mean mass of 2.6g, then the percentage transmitter mass to body mass ratio of the threshold limit was 10.4%. Transmitter mass to body mass ratios have been shown to influence a variety of animals in different ways. Lutterschmidt (1994) showed a reduction in the speed of movement of garter snakes with implanted transmitters when the tags were 15% of the body mass or above. Tags weighing 10% of body mass or less gave no measurable reduction in the speed of movement of garter snakes. Many of the published studies on effects of tagging are on birds (e.g. Greenwood and Sargeant 1973, Amlaner *et al* 1979, Warner and Etter 1983). In all these studies the higher the transmitter mass to body mass ratio the greater the effect on the studied variable.

In all three years the within treatment variability of mass was very high. In 1993 and 1994 this was explained by differences in the hydration states of *B. bufo*. In 1995, *B. bufo* hydration state was controlled for, yet the mass remained very variable. When placed in water for prolonged periods of time, some animals would defecate and this resulted in up to a gram difference in mass. In future investigations, collection and weighing of faeces may control for this variable. The investigations were conducted over a comparatively short period of time, differences in mass would increase if the investigation continued for several months. Reduction in feeding rate is a much less variable indicator of *B. bufo* stress over a short term investigation.

White and Garrott (1986) suggest that tagging will always have some effects on the study species. Before any tracking begins the method of tag attachment should be tested to determine the effect of tagging on the variable to be measured in the field. In most cases, this is impractical, as radio-tracking is used because no other method of measurement is possible. It is therefore necessary to devise a method of comparing tagged and untagged animals in a meaningful way, by using indicator variables such as mass and feeding rate. The choice of indicator variable should depend on the study species, the field variable/s chosen and the method of attachment.

2.5 CONCLUSIONS

- Indicator variables (mass and feeding rate) demonstrated detrimental effects of tag attachment.

- Implantation of transmitters is not suitable for female *B. bufo* as this method resulted in a significant reduction in feeding.

- Ingestion of transmitters may cause reduction in the amount of field data in long term studies as regurgitations were common in small male *B. bufo*.

- In radio-tracking studies transmitter to body mass ratio should be less than or equal to 10%. In male *B. bufo* with body mass of less than 25g, tagging by ingestion, with tags of mean mass 2.5g, caused a reduction in feeding rate and loss of body mass.

3: MONITORING OF HABITAT USE BY RADIO-TRACKING

3.1 BACKGROUND

Hutchinson (1957) defined a species niche as a multidimensional hypervolume which can be described by all the interactions of an organism and its environment. One of the most important aspects of species niche is habitat use and preference. Habitat preference is best defined as the likelihood that a resource will be chosen if offered on an equal basis to others (Johnson 1980). Selection can be defined as the process by which an animal chooses a resource (Johnson 1980) and selectivity studies involve the comparison of resource use with availability (Thomas and Taylor 1990).

The aquatic habitats of amphibians have been well studied and aspects of aquatic habitat which influence the presence or survival of amphibians include: pesticide pollution (Cooke 1981) presence of fish (Banks and Laverick 1986, Oldham and Swan 1991), invertebrate density (Banks and Beebee 1987, Pearman 1995), ionic content (Beebee 1979, 1981, Hecnar and M'Closky 1996), temperature (Beebee 1983a, Crawshaw *et al* 1992) vegetation cover of pond (Beebee 1981, Oldham and Swan 1991), pH (Cummins 1989, Beebee *et al* 1990) and the presence of predatory or competing species of amphibian (Alford and Wilbur 1985, Griffiths 1991, Griffiths *et al* 1991, Tejedo 1991).

The terrestrial habitats have been less well studied, yet there is evidence that terrestrial habitat has a significant influence on aspects of population structure and dynamics in amphibians. The body size and sexual maturation rates of *R. temporaria* can alter with different terrestrial habitats (Augert and Joly 1993). The species richness of amphibian communities alters with habitat type and degree of fragmentation (Joly and Morand 1994). Altering terrestrial habitats by clear-felling, reduced terrestrial amphibian populations by up to 70% in the coastal forests of Canada (Dupuis *et al* 1995). *B. bufo* size at breeding and therefore fecundity in females, was influenced by the juvenile terrestrial environment (Swan 1986). There is also some indirect evidence to suggest that sexually mature *B. bufo* show a high degree of fidelity to their first year habitat choice (Swan and Oldham 1989).

Terrestrial habitat choice and availability are important in the population structure and dynamics of *B. bufo*. Despite this there has been little published work on the habitat preference and requirements of *B. bufo*. Most work to date on the habitat preference of British Bufo species has centred on the vegetation surrounding the breeding sites (e.g. Beebee 1977b, 1979,1981) with no direct measurement of densities in different habitats or estimates of habitat preference. The literature generally demonstrates a requirement for scrub, woodland or rank/ rough grassland in the terrestrial habitat of *B. bufo*. *B. bufo* trapped in scrub and woodland tend to be in good condition (from mass to length ratios) indicating that these are high quality habitats, when compared with arable and pasture (Swan and Oldham 1989, Beebee 1981, Cornish 1992). *B. bufo* is often absent in areas of exclusively arable farming, where hedgerows, woodlands and scrub have been removed (Beebee 1977b).

The importance of scrub and woodland were clearly demonstrated by the removal of ungrazed scrub from the proximity of a breeding site in Leicestershire which resulted in the decline of *B. bufo* numbers (Oldham and Swan 1991). Areas of scrub and woodland at the same breeding site supported consistently higher densities of *B. bufo* compared with arable and pasture. Contrary to the majority of published data Strijbosch (1980) found *B. bufo* showed a preference for open habitats such as arable and pasture fields.

B. bufo are nocturnal during the summer months, and spend much of the day in refugia, however most afore mentioned studies only measured habitat use by active animals or they do not differentiate between refugia habitats and habitat use by active animals. Denton and Beebee (1993) investigated the summer refugia of *B. bufo* and *B. calamita*, finding *B. bufo* in animal burrows or under litter and logs but the results were based on only eight individuals from sand dune and heath sites.

Most of the literature therefore indicates a preference for woodland and scrub. The available data are however very scarce and do not demonstrate which is or are the most preferred habitat/s of *B. bufo*. The question of what abiotic and biotic factors mediate habitat preference also remains unanswered. To answer these questions detailed studies of individual *B. bufo* are required to determine changes in habitat preference with time, abiotic and biotic factors.

Since *B. bufo* are mainly nocturnal/crepuscular, small and cryptically coloured, locating them can be a major obstacle in such detailed studies. Radio-tracking solves the problem of locating the animals. In the present study radio-tracking, using a combination of automated and hand-held systems, was only conducted in summer, as only non-migratory habitat choice was investigated. In spring, *B. bufo* migrate *en masse* to breeding sites and in autumn *B. bufo* make small pre-hibernation migrations towards the breeding sites (Denton and Beebee 1993, Sinsch 1988) tracking was therefore limited to May - September.

3.2 THE TRACKING AREA

Inadequate knowledge of the available habitats is one of the major criticisms of habitat preference studies (Alldredge and Ratti 1986, Johnson 1980, Thomas and Taylor 1990). Therefore, in this study all tracking was conducted within an enclosed area, ensuring the habitats available to the study animals were known which overcame the problems caused by unknown or undefined habitat areas. The main study site is situated in a rural garden near Coleorton (SK396168) in north-west Leicestershire, and the main study population has been extensively studied and is described by Oldham and Swan (1991). This site was chosen as it offered a range of habitat types and was a suitable terrestrial habitat as it supported a population of *B. bufo* prior to the current study.

The radio-tracking enclosure was surrounded by a chicken wire fence (mesh size 1cm) with a 50cm internal and external overhang to reduce migration of the local population of *B. bufo*. A pair of pitfall traps consisting of ten litre buckets, one on the inside of the enclosure and one on the outer side of the fence, were placed every 20m along the perimeter. The external pitfall traps caught *B. bufo* of the local population which were migrating into the enclosure. The internal pitfall traps were established six months prior to radio-tracking in an attempt to catch the population of *B. bufo* already established within the enclosure.

Habitat categories should be kept to a minimum to reduce type II errors (Alldredge and Ratti 1986). Small numbers of broad habitat categories would also allow comparison with other studies and facilitate the production of general habitat recommendations. The radio-tracking area was 39m by 28m at the widest points and consisted of four habitats managed

to simulate those commonly occurring in agricultural landscape. The four main land use types within the enclosure were:

A: Woodland B: Rough grassland

C: Arable/Cultivated D: Improve Pasture

The habitats were divided by a box hedge and the arable area was enclosed on two sides by a strip of uncultivated land or 'edge'. This edge represented a linear land feature such as headland and hedge/ditch. The flora in the radio-tracking enclosure habitats was surveyed in May 1995. A list of species obtained by this quadrat-based floristic survey is given in Appendix A. The survey methods are also given in Appendix A. A brief description of each habitat is given below.

The woodland within the area was dominated by a large oak and several small ash and elm trees. The woodland area was shaded by both deciduous and coniferous trees rooted outside the enclosure, providing a rich ground litter. The woodland ground layer consisted largely of ivy and grasses with a small patch of black-currant bushes on the central edge of the habitat. The rough pasture was an ungrazed, unimproved grassland dominated by the false oatgrass (*Arrhenatherum elatius*), nettles (*Urtica dioica)* and broad leaved dock (*Rumex obtusifolius*). The arable area was tilled yearly, organically enriched and planted with brassicas and other vegetable crops. No herbicide was applied to the site during tracking and as a result large numbers of ruderal/arable weeds encroached into the main habitat in 1994. The rye-grass (*Lolium perenne*) pasture was planted and fertilised in 1990 and had established a species poor turf. To maintain the low diversity of the sward, the pasture was cut and clippings removed to simulate grazing. To prevent the encroachment of broad-leaved docks and ruderal weeds into the arable a herbicide was applied at the end of tracking in 1993 and 1994.

The two most preferred habitats in 1993 and 1994 were on the southern side of the enclosed area. To determine whether *B. bufo* were choosing habitat and not demonstrating a directional preference the relative positions of the tracking area habitats were altered between 1994 and 1995 from those shown in Figure 3.1 to those shown in Figure 3.2. It was practically impossible to move the woodland successfully so this habitat was left undisturbed. The rough grassland of 1993/4 was mown, and the top layer of seeds and rough grass turf removed. The soil was then rotovated and organically enriched. This

cultivated soil was planted with brassicas and other vegetable crops over half of the area and the other half left as rotovated soil to simulate crop fields.

The 1993/4 improved pasture was allowed to grow and seed at the end of tracking in 1994. This increased the species number in the sward, which were further enriched by additions of the turf from the old rough grassland and fresh hay from a species rich hay meadow. The final alteration was to convert the old arable area to an improved grassland. This area was rotovated, flattened and seeded with a horse pasture mix. To ensure a species poor turf was cultivated, a broad-leaved herbicide and an NPK fertiliser were applied in winter 1994 and the herbicide applied again in the spring of 1995. The high rainfall in early 1995 ensured that a thick, species poor sward grew in time for tracking in 1995. The improved pasture was then mowed on a regular basis and the clippings removed to simulate grazing.

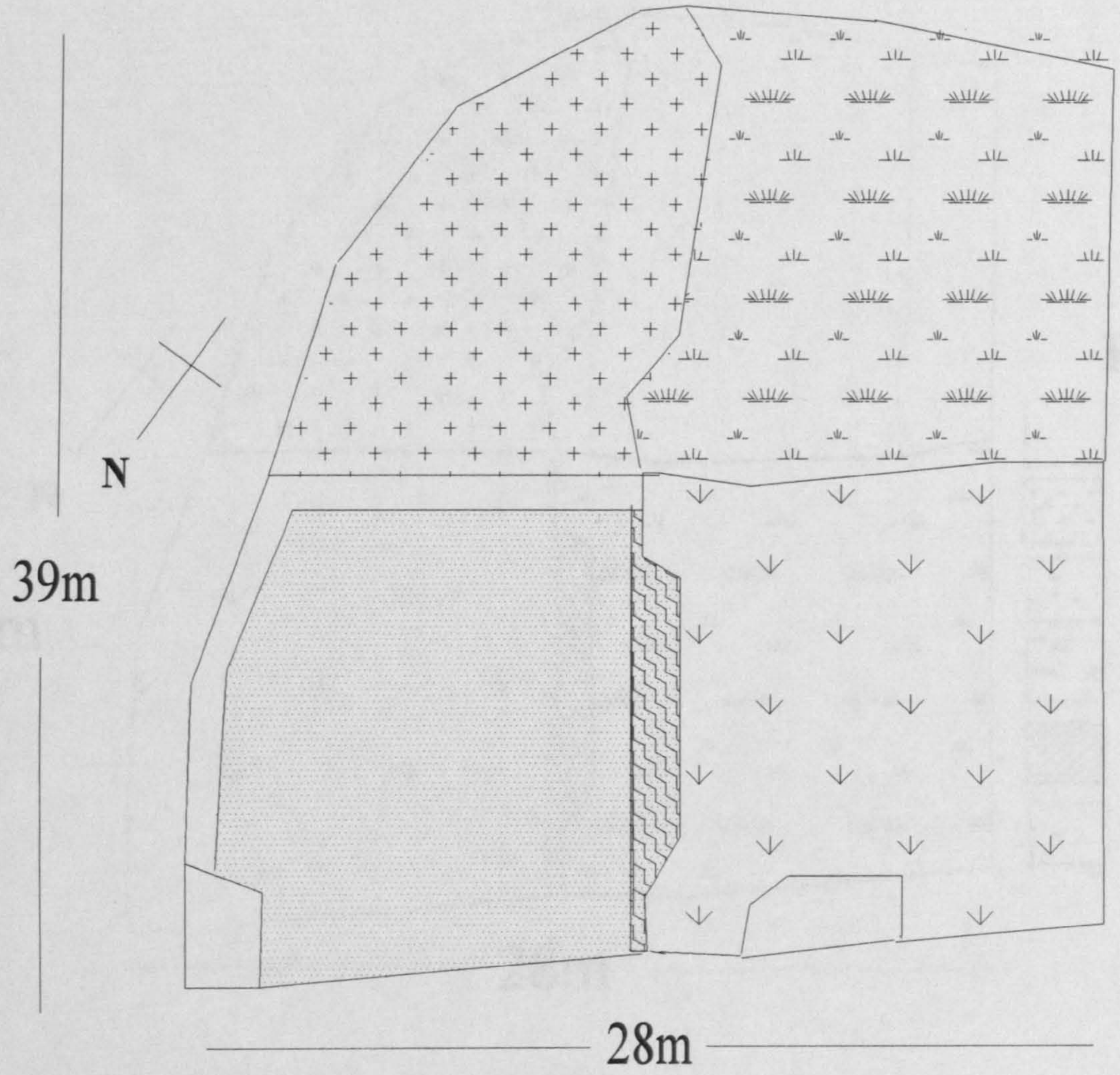
3.3 PRELIMINARY STUDIES

3.3.1 The Automated Radio-tracking System

The radio-tracking investigations were initially conducted using an automated system already in place in the Coleorton site (French *et al* 1992). The system consisted of two three-element Yagi antennas attached to rotating bases connected to the control station by low-loss cables. A computer tunes the VHF receiver via an interface and the receiver was connected to two control boxes via a second interface. The system could be operated manually but normal data collection was controlled by software. A complete system description is given in French *et al* (1992). The system was operated in conjunction with Biotrack (Wareham, Dorset, UK) S2 radio transmitters. Initial automated tracking began on November 12th 1992 and finished on September 17th 1993. No *B. bufo* were tracked between 3rd December 1992 and 19th March 1993 to allow for hibernation and breeding migration. No automated radio-tracking took place between the 9th June and 11th August 1993 whilst the system was under repair.

Figure 3.1

Radio-Tracking Area - 1994



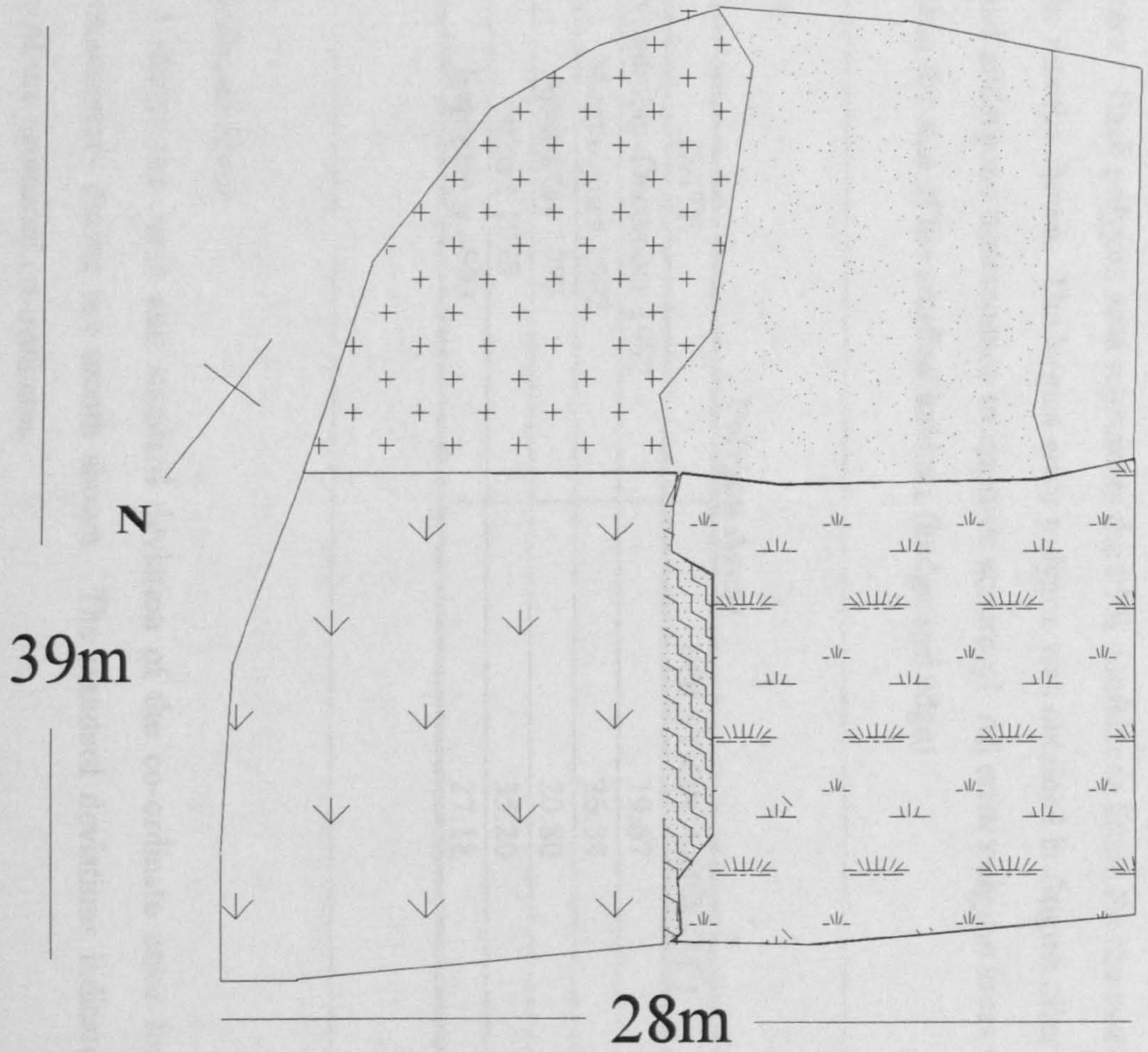
Habitat Type

- Linear land features (4.8%)
- Woodland (19.0%)
- Improved Pasture (23.9%)
- Rough grassland (27.6%)
- Hedge (5.7%)
- Arable (19.0%)

See text for descriptions of habitats and changes made from 1994 to 1995.
Figures in parentheses are the percentage of total radio-tracking area.

Figure 3.2

Radio-tracking Area - 1995



Habitat Type

- Linear land features (4.4%)
- Woodland (19%)
- Improved Pasture (25.6%)
- Rough grassland (24.7%)
- Hedge (4.4%)
- Arable (22.2%)

Descriptions of habitats and details of habitat changes from 1994 to 1995 are given in text. Figures in parentheses are the percentage of total radio-tracking area.

3.3.2 Evaluation Of Automated Radio-Tracking Data

The performance and accuracy of the radio-tracking system was monitored using control tags placed at known co-ordinates. Error polygons were derived from these data following calculations described in Kenward (1987). The automated tracking system was used in conjunction with a hand held three element Yagi antenna and a portable receiver (Mariner Radar, Lowestoft, Suffolk, UK). *B. bufo* were located 2-5 times per week in the daytime using the hand held system. Co-ordinate error was then calculated as the difference between the estimated location given by the system and the actual position of the animal as recorded using the portable receiver. Data were analysed using the Ranges IV software (Kenward,ITE, Wareham, U.K.) to calculate home range data and percentage area of habitat in the radio-tracking enclosure. The data analysis is described in Kenward (1990).

3.3.3 Results Of Preliminary Trials Of Automated System

(i) Radio-tracking System Error

Table 3.1 gives the error polygons calculated from the bearing errors of the control transmitters. Each polygon area represents the 95% confidence limits for the system during the months shown. The largest error polygon was obtained in August after the system had undergone maintenance to improve accuracy! All error polygon areas were greater than the size of the smallest habitats (hedge and edge)

Table 3.1

Polygon Areas

DATE	POLYGON AREA (M^2)
November-December 1992	19.67
March-April 1993	26.59
April-May 1993	20.80
August 1993	35.20
September 1993	27.18

(ii) Co-ordinate Error

Table 3.2 shows the mean and standard deviation of the co-ordinate error for the control transmitters during the month shown. The standard deviations indicate the reliability of the estimated co-ordinates.

Table 3.2

Mean and Standard Deviations of Co-ordinate errors from November 1992 to September 1993.

MONTH	MEAN X Co-ordinate (m)	STANDARD DEVIATION X Co-ordinate (m)	MEAN Y Co-ordinate (m)	STANDARD DEVIATION Y Co-ordinate (m)
NOV 1992	- 6.1	21.2	7.5	67.3
DEC 1992	16.4	48.8	- 39.2	42.4
MAR 1993	- 2.3	19.9	29.2	147
APR 1993	16.0	46.8	13.8	65.5
MAY 1993	- 1.4	33.9	- 4.4	64.4
JUNE 1993	-19.2	73.5	- 16.4	152.6
AUG 1993	- 2.9	43.1	- 9.65	88.3
SEPT 1993	0.6	47.1	3.5	220.0

(iii) Home Ranges Sizes and Interfix Distances.

Table 3.3 gives the total number of Outer Convex Polygon home ranges and interfix distances for each *B. bufo* tracked. Home range estimates of between 5.6 x 10^{-2}Ha to 9.0 Ha reflect the error inherent in the estimation of co-ordinates by the automated system and not the true size of *B. bufo* home ranges. Due to the large error in the estimation of home ranges, accurate calculation of the habitat use by *B. bufo* was impossible from the automated tracking data.

3.3.4 Automated Radio-tracking: A Summary.

The results in 1993 proved that the automated system needed redesigning before automated tracking could be used for estimation of habitat preference of amphibians. The redesigned system was to include four antennae to allow accurate cross-triangulation and the incorporation of more sophisticated programmes for the estimation of radio-fix co-ordinates. Two further antennae were wired to the system and the software programme re-written to include estimation of co-ordinates by maximum likelihood estimator described in White and Garrott (1986). The control of the four antennae required new custom made hardware which was still at the debugging stage at the completion of this project in Autumn 1995. Due to the lack of reliability of the automated system, all tracking in 1994 and 1995 used only the hand held system

Table 3.3

Outer Convex Polygon Estimates of Home Range Size of Radio-tracked *B. bufo* In a 0.1Ha Enclosure - Summer 1993

MALES			FEMALES		
B. BUFO	OCP home range (Ha)	Interfix Distance (m)	*B. BUFO*	OCP home range (Ha)	Interfix Distance (m)
AM1(a)	0.98	44.69	AF1	0.15	13.84
(b)	1.16	29.49			
AM2	0.12	16.17	AF2	5.98 x 10-2	16.20
AM3	5.56×10^{-2}	16.20	AF3	0.11	10.96
AM4	0.54	22.16	AF4	0.59	37.22
				0.65	31.55
AM5	0.40	22.66	AF5	0.68	37.22
				1.21	31.55
AM6	0.13	25.67	AF6	0.21	16.92
				0.54	19.23
				0.39	23.43
AM7	0.68	34.18	AF7	0.38	14.87
AM8 (a)	0.56	31.59	AF8	0.12	21.18
(b)	0.66	24.66		0.17	12.46
AM9	0.49	31.10	AF9	0.18	14.68
				0.13	14.39
AM10	0.45	32.75	AF10	0.14	22.13
				0.13	17.03
AM11	7.79	138.35	AF11	9.00	107.69
AM12	0.49	46.36	AF12	0.34	32.64
AM13	0.48	22.93	AF13	0.67	51.14
AM14	0.27	52.74	AF14	0.45	35.82
AM15	1.00	67.25	AF15	0.61	44.59
			AF16	1.23	72.43
			AF17	1.75	78.29
			AF18	1.82	52.41

The OCP (Outer convex polygon) home ranges and the interfix distances were calculated using the automated radio-tracking system. OCP's give an indication of the home range size of each individual. Interfix distances represent the mean distance travelled between each fix and are therefore an indicator of the speed of the movement of each individual. Fixes were made at approximately 60 minute intervals per day and there were approximately 12 hours between each days recording.

3.4 RADIO-TRACKING USING THE HAND HELD SYSTEM

Data from the automated system was too inaccurate to provide habitat preference estimates. Automated radio-tracking could not provide data on the behaviour of animals when in different habitats. Therefore a hand held system was used to obtain accurate data on the habitat use by *B. bufo* and the activity of *B. bufo* whilst in different habitats. These data were used to produce estimates of habitat preference of *B. bufo* by comparing habitat use with availability within the radio-tracking area. Preferences of males and females were compared to determine any sex-linked differences in habitat preference. Diversity of vegetation in the microhabitats chosen by *B. bufo* was recorded to determine any microhabitat selection.

Some species of amphibian show fidelity to the same breeding site from year to year (e.g. Reading Loman and Madsen 1991). *B. bufo* have been shown to return to the same home range after the spring migration (e.g. Haapanen 1974, Sinsch 1988). Habitat preference may therefore be determined by genetic or learned fidelity to specific habitat type/s. The radio-tracked *B. bufo* were chosen from two discrete populations which had little or no interbreeding and were from markedly different habitats around their respective breeding ponds. The habitat preference of radio-tracked *B. bufo* from the two populations were compared to assess the importance of habitat fidelity.

3.4.1 Methods

(i) Capture And Release Of Animals

Animals were caught during the spring migration in each of the three years. Animals were checked for visible signs of infection (skin lesions etc.) and those which appeared healthy were given marks specific to their site of origin using a Panjet. The origin of *B. bufo* was separated into two categories; those from the local population at Coleorton (SK396168) were designated "local" those from populations over 5 Km away were designated "foreign". Local and Foreign *B. bufo* were kept in separate vivaria. Immediately prior to radio-tracking the snout-vent length, hydrated mass and site of origin of each animal was recorded. Different individuals were used for each radio-tracking period. Animals were chosen based on size (snout-vent length and mass), the mass limits for radio-tracking by ingestion were described in chapter 2. Biotrack S2 transmitters used in the

current study had a mean mass of 2.5g until May 1994 when manufacturer modifications reduced the mean mass to approximately 2.0g. Animals of greater than 25g were tracked before May 1994 and greater than 20g after May 1995 to ensure that transmitter mass to body mass ratio never exceeded 10% (chapter 2). After each radio-tracking period, transmitters were removed and animals were returned to their vivaria for at least 48 hours. Animals were observed to establish they were feeding and if any visible signs of damage to the skin were detectable. Animals which appeared healthy were released at their site of origin.

(ii) 1993

Between four and eight *B. bufo* were tracked at any one time. A total of 15 males and 18 females were tracked in 1993. Initially *B. bufo* were only tracked once per week as a back up to the automated system. After June the number of locations was increased to 3-5 per week. The co-ordinates, habitat choice and vegetation at the location of *B. bufo* were recorded. The vegetation was recorded within a quadrat ($1m^2$) as percentage cover of ground cover groups:

Unvegetated ground,

Moss and lichens,

Tall herbs and grasses,

Woody perennials

Ruderal weeds/cultivated plants.

Thirty random number co-ordinates were used to locate 30 $1m^2$ quadrats throughout the enclosure. On 20th July 1993 the ground cover groups in each of these quadrats were recorded. *B. bufo* were weighed on release and once every 14 days after release using a digital field balance. All data from 1993 were collected diurnally when *B. bufo* were in refugia.

(iii) Tracking Protocol in 1994 And 1995

In 1994 and 1995 *B. bufo* were tracked both diurnally and nocturnally to obtain data on both refugia preference and habitat preference of active animals. All fixes when *B. bufo* were out of refugia were recorded as "active" fixes and all those when *B. bufo* were in refugia were recorded as "passive" fixes. A refugium was defined as any location where *B. bufo* was completely covered and inactive. In practice identification of refugia was simple

as over 70% were underground burrows the remainder were above ground under logs, bricks or leaf litter. *B. bufo* were released and given 24 hours to acclimate to the radio-tracking enclosure. Between 10 and 12 *B. bufo* were tracked at any one time, to give a maximum density of 120 *B. bufo* per hectare. *B. bufo* were always released at the release point (approximately at the centre of the enclosure) facing random directions. A total of 15 males and 15 females were tracked in 1994 and a total of 20 males and 20 females in 1995.

B. bufo were tracked a minimum of 3 nights per week from just after sunset until just after sunrise or until *B. bufo* had become passive for a minimum of three hours. *B. bufo* were located once per hour and observed through a red filtered torch for three minutes. *B. bufo* behaviour over the three minutes was recorded in one of three groups; "sitting", "moving" and "feeding". The sitting category included all above ground activity where *B. bufo* were not altering location or feeding. Orientation towards moving prey that only involved rotation of *B. bufo* was included in this category. The moving category included all times when *B. bufo* were walking across the habitat without hunting prey and this was recorded if it occurred any time during the three minute observation period as long as feeding was not observed. Feeding involved all hunting of prey during which *B. bufo* changed location and was recorded for any three minute observation period during which it occurred. *B. bufo* were weighed on release and once every two weeks using a digital field balance.

3.4.2 Analysis

(i) 1993

To asses microhabitat selection by *B. bufo*, diversity of vegetation at the location of the radio-tracked *B. bufo* was compared with diversity of the radio-tracking enclosure. A Shannon-Weaver index of diversity was calculated for the habitat category data collected in 1993. H' value was calculated for each of the $1m^2$ quadrats as follows:

$$H' = -\sum_{i=1}^{s} Pi \ln Pi$$

Where P_i = proportion of the ith ground cover group. The H' values for the random quadrats were compared with those of the *B. bufo* locations using the Mann-Whitney U test.

(ii) Behaviour And Mass Data

The behavioural data for each *B. bufo* was converted to percentages of total number of fixes per *B. bufo* spent in each activity. The percentage number of fixes spent moving by males were compared with those of females in 1994 by a Kruskal-Wallis non parametric oneway ANOVA. A second Kruskal-Wallis was used to compare data from males and females in 1995. The logs of the initial and final mass of each *B. bufo* from the three years combined were compared using a paired T-test.

(iii) Calculation Of Sampling Increments

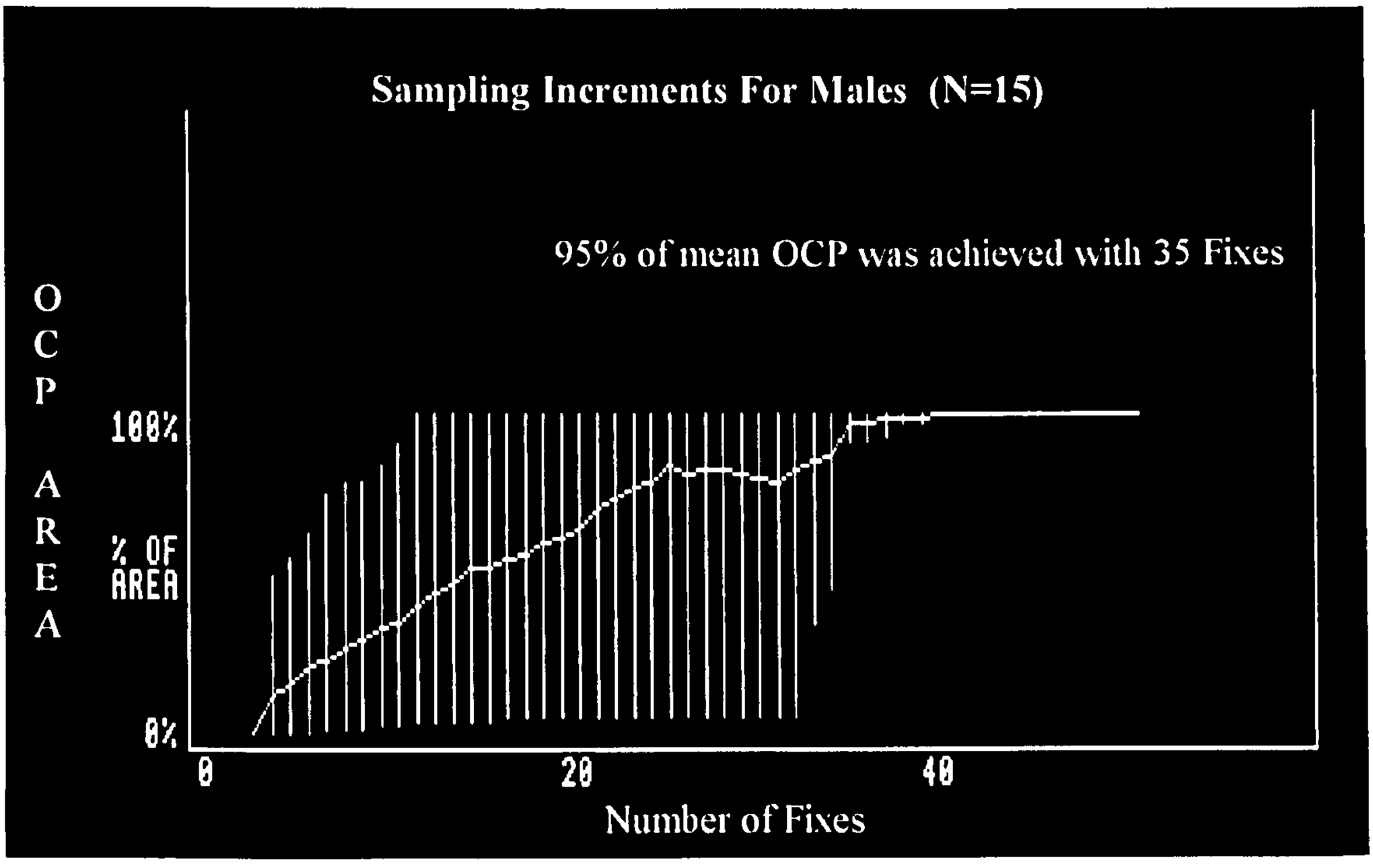
Fixes of *B. bufo* outside of refugia (active fixes) during 1994 were used to produce outer convex polygons (OCP) using "ranges IV" as described in Kenward (1990). These were then plotted against the number of fixes to give plots of sampling increments. The mean sampling increment plots for males and females with max-min drop lines are given in Figure 3.3a and b. The number of fixes giving 95% of the mean OCP area were used as the minimum fix number. All animals with less than the minimum number of fixes are excluded from habitat preference analysis described below. Table 3.4 shows the number of animals of each sex, from each origin eligible for statistical analysis.

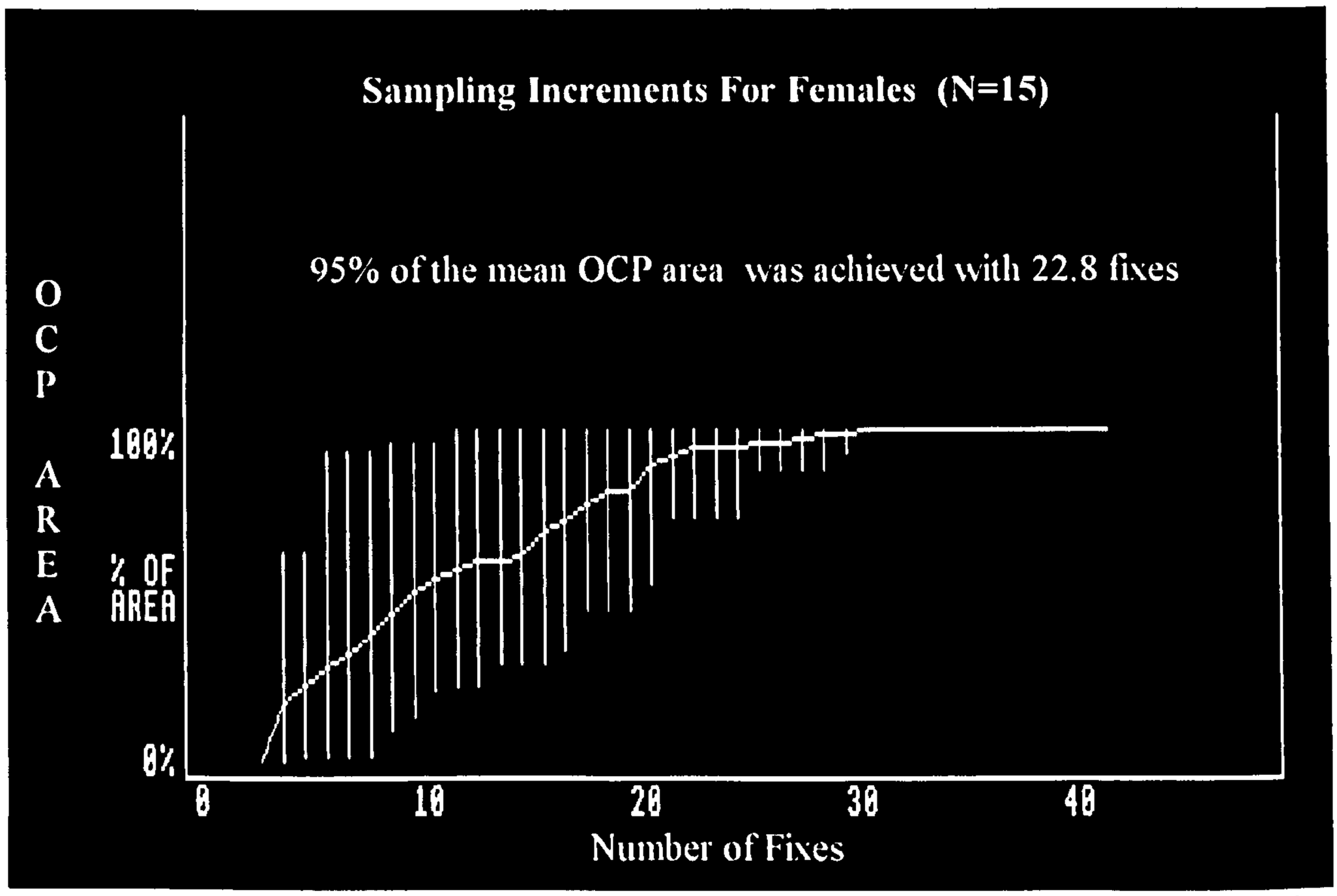
(iv) Data Transformation

Compositional analysis was used to convert radio-tracking data to allow habitat preference to be assessed. The proportion of fixes per habitat can not be used in normal statistics as they automatically sum to one and are therefore not independent. In order for parametric statistics to be applicable the data must be rendered independent and normal by the use of transformation. Aebischer *et al* (1993) describes a method of transformation specifically designed for radio-tracking data. A ratio of percentage fixes per habitat with edge habitat as the denominator was calculated, thus ensuring independence (by removing unit sum constraint). A ratio of the percentage areas of the enclosure habitats with area of edge as the denominator was used to represent the available habitats. The ratios were then logged and checked for normality. These converted data are termed log-ratios.

Figure 3.3

Mean sampling Increments For Radio-tracked *B. bufo* - 1994





Outer convex polygons (OCPs) are estimates of home range size made from radio-tracking data. The main plot lines are the mean value of all 15 *B. bufo* the drop lines show the range. The number of fixes which gave 95% of the mean OCP area (Y axis) were used as the minimum number of fixes. All animals with less than the minimum number of fixes were excluded from analysis of habitat preference.

Table 3.4

Number Of Animals Used In Calculation Of Habitat Preference.

YEAR	SEX	ORIGIN	Number Of Animals
1994	Male	Local	7
		Foreign	8
	Females	Local	7
		Foreign	8
1995	Male	Local	8
		Foreign	7
	Females	Local	10
		Foreign	10

Calculation of the minimum number of fixes from sampling increments was described in section 3.4.2 (iii). Only animals with greater than the minimum number of fixes were used in statistical analysis of habitat preference.

(v) Habitat Use And Preference

The log-ratio difference values from refugia habitats were compared with those of active *B. bufo* using a paired T-test. The first calculation is to determine whether habitat use is non-random. The log-ratio of percentage habitat availability was subtracted from the log-ratio percentage habitat use (percentage number of fixes) and the difference between these log ratios was calculated. If habitat use was random then habitats will be used in proportion to their availability and the mean log-ratio differences would be zero. Two 1-sample Hotellings T^2 tests were conducted, one on the active data and one on the passive data. These tested if the mean log-ratio differences from the current study were significantly different to those that would be obtained with random habitat use (a mean of zero). Years(1994 and 1995) and sexes (males and females) were pooled for the test of non-random habitat use.

The log-ratio differences were then entered into a three-way multiple analysis of variance (MANOVA) with sex, (male/female) year (1994/1995) and origin (local/foreign) as the independent variables. The dependent variables were the log ratio differences for each of the following habitats: hedge, woodland, arable, pasture, rough grassland. The same three-way MANOVA design was used for analysis of active and then the passive habitat use data. Finally where non-random habitat use was demonstrated, pair-wise comparisons were made to determine the order of preference from most to least preferred. The ratio of the mean pair-wise log-ratio differences to the standard error of the mean is a T value measuring departure from random. To

control for an increase in type 1 error from multiple testing (multiple calculations of T values) a modified Bonferroni correction (Simes 1986) was applied to the data.

In the Bonferroni correction the test α' is the calculated by choosing the nominated α (usually 0.05) and dividing it by n, where n is the number of tests performed. The Simes version of the Bonferroni correction ranks the P values from smallest (most significant)P_0 given a rank of 0 to highest (least significant)P_n given a rank of n-1. The symbol for ranks = k. In the Simes modified Bonferroni procedure α' changes with the ranked P values from α_o'to α_{n-1}'. The test with the smallest P value (most significant) is calculated first

$$\alpha_o' = \alpha/n.$$

If $P_0 < \alpha_0$' and the null hypothesis is rejected, then the second smallest value of P (P_1) can be compared to α_1' which is calculated as follows:

$$\alpha_1' = \alpha /n-1.$$

This procedure is repeated comparing each P value (P_0 ... P_{n-1}) in order of rank from 0 to n, with an α' value calculated by dividing α by the n-k. Where k is equal to the rank of the P value. When $P_x > \alpha_x$' and the null hypothesis is not rejected, then the calculation stops and all values of P greater P_x are not significant at α. This method does not increase the Type I errors above α but reduces Type II errors compared with the classical Bonferroni procedure.

3.5 RESULTS

3.5.1 1993 Habitat Use And Survival Of Radio-tracked *B. bufo*

The minimum number of radio-tracking fixes was 35.0 for males and 22.8 for females. Figure 3.4 shows the total number of radio fixes in each habitat from 1993. Most of the 1993 fixes are for refugia and are of passive animals.

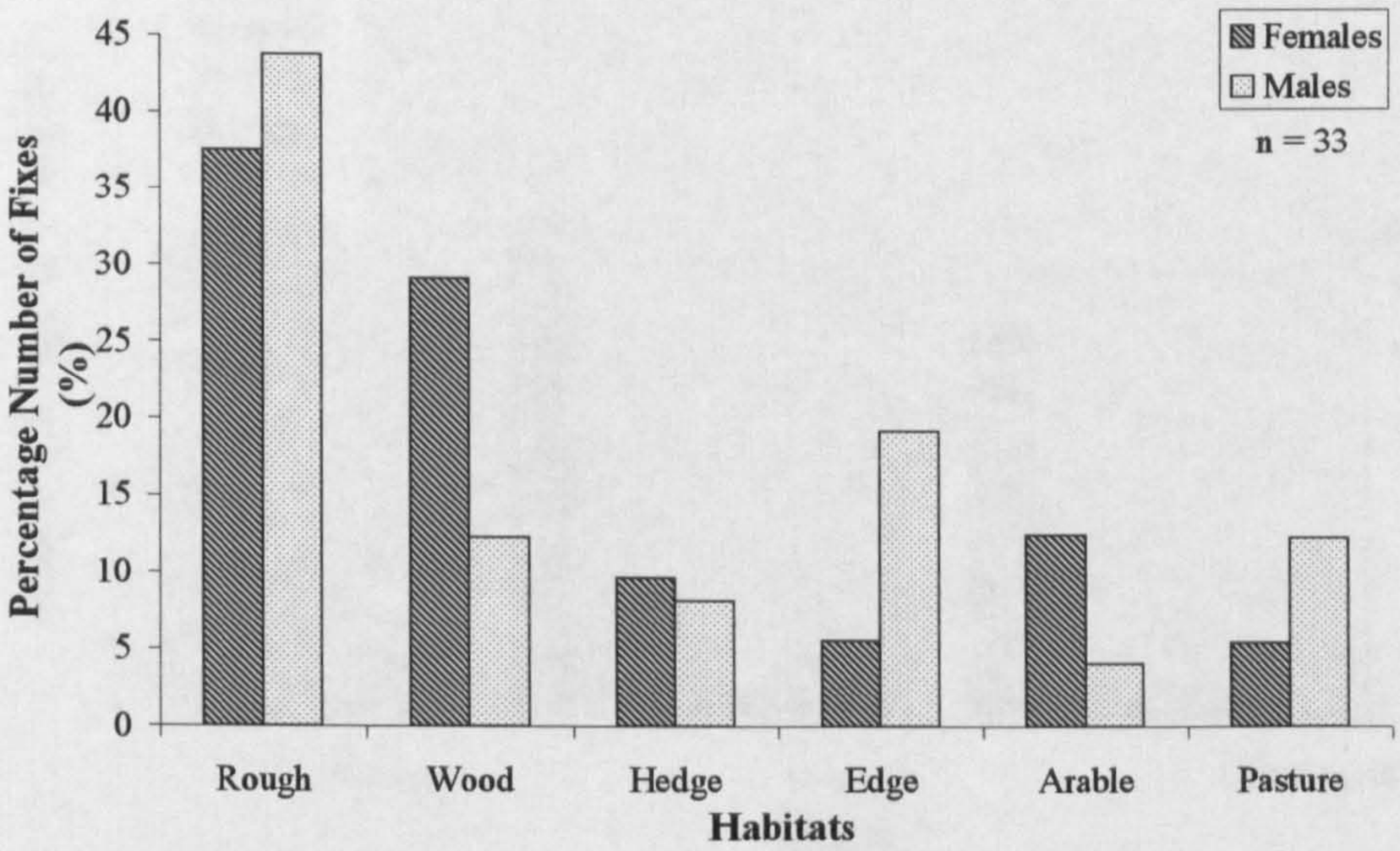
The order of habitat use from most used to least used is as follows:

Rough grassland> woodland>arable>hedge>pasture>edge for females

Rough grassland> edge>pasture>woodland>hedge>arable for males

Figure 3.4

Habitat Use By Radio-tracked *B. bufo* - 1993



A total of 33 *B. bufo* were radio-tracked in 1993 within a fenced radio-tracking area.
Rough = Rough grassland habitats Wood = Woodland. Hedge = Box hedgerow.
Arable = Cultivated area with non-cereal crop plants. Pasture = Simulated improved pasture.
Edge = Linear land features at the edge of the fenced area.
The radio-tracking area is described more fully in section 3.2.

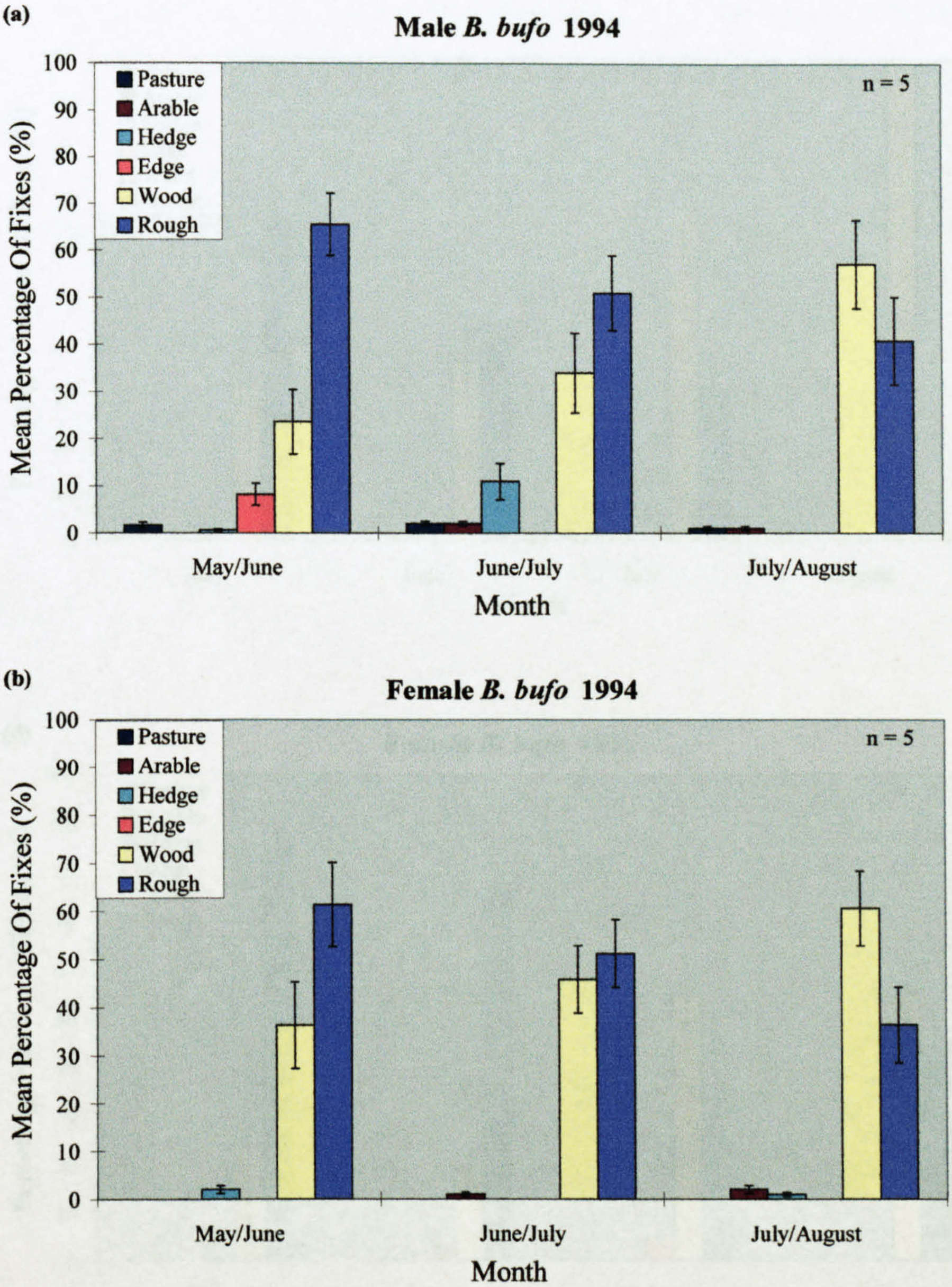
No conclusions can be drawn from these data as only two animals had greater than the minimum number of fixes in 1993. Two of the 33 tracked animals were recovered, two fell prey to birds and 18 animals regurgitated transmitters resulting in the loss of these individuals. Predation of tracked *B. bufo* is discussed in Section 6.11. The fate of the remaining animals is unknown probably due to transmitter failure. The Shannon-Weaver index of habitat diversity for quadrats at *B. bufo* locations differed significantly from that at random locations ($Z_{1,50}$= -3.33, P<0.001). Vegetation at *B. bufo* locations had a mean diversity index of 0.80(+/-0.19) and vegetation at random locations had a mean diversity index of 0.52 (+/-0.26).

3.5.2. Refugium Habitats 1994 And 1995

Figures 3.5(a-d) show the use of refugia by male and female *B. bufo* in 1994 and 1995 respectively. Woodland and rough grassland were the most used refugia habitats in both years by both sexes. There is a movement from rough grassland as the main refugium habitat in May to woodland in August.

Figure 3.5

Refugium Habitats Used By Radio-tracked *B. bufo*

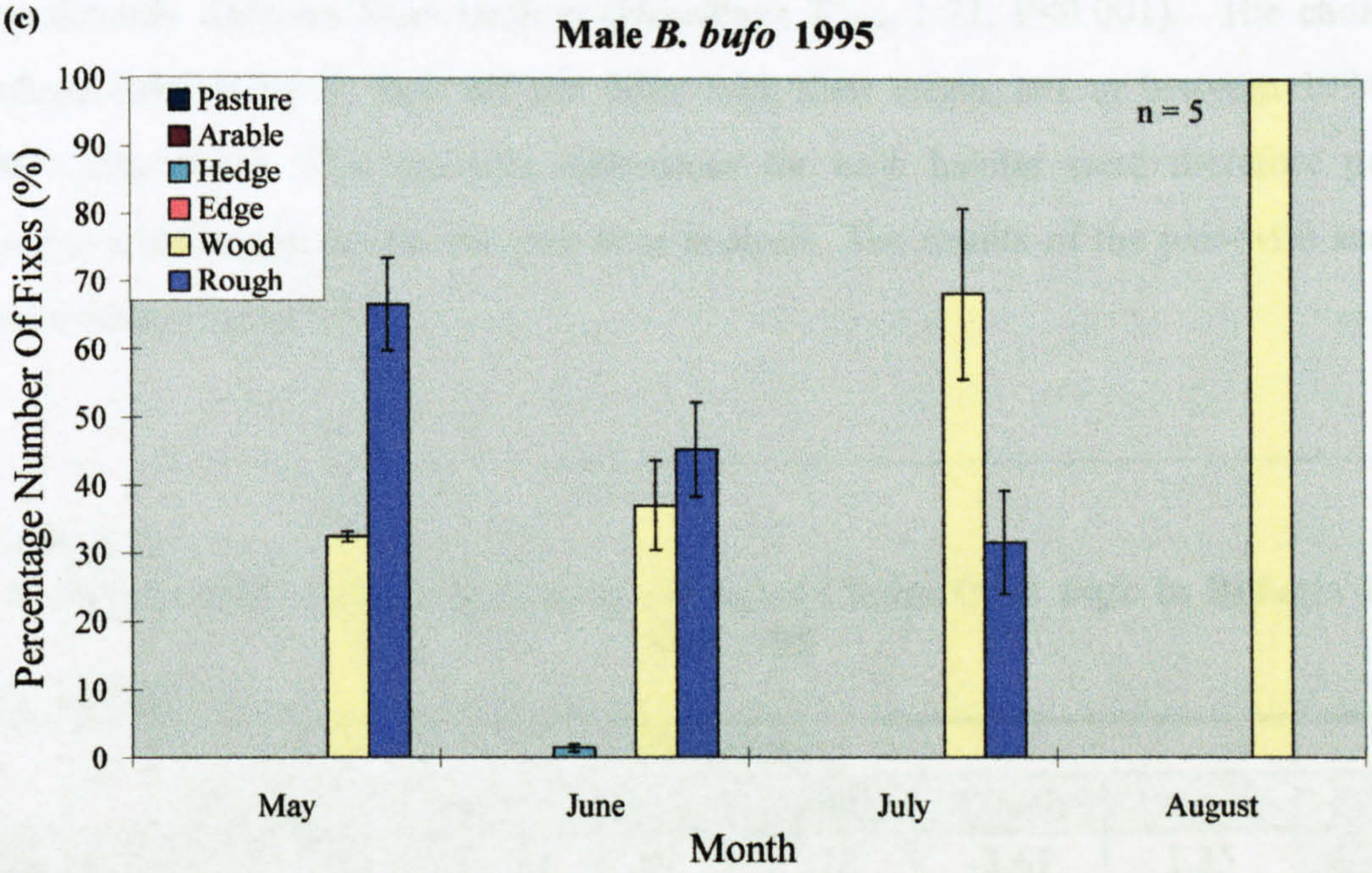


Tracking Periods
Ma/Ju = 6th May to 6th June.
Ju/July = 7th June to 16th July.
Jy/Aug = 18th July to 24th August

Data shown are the mean percentage of fixes from the five animals tracked (n = 5) in each of the three tracking periods. Error bars are +/- one standard error of the mean.

Figure 3.5 (cont.)

Refugium Habitats Used By Radio-tracked *B. bufo*



Data shown are the mean percentage of fixes from the five animals tracked (n = 5) in each of the four tracking periods. Error bars are +/- one standard error of the mean.

Habitats used for refugia and habitats used by active animals differed significantly ($T_{1,324}$=3.58,P<0.01) so data were analysed separately. Refugium habitat choice was significantly different from random (Hotellings $T^2_{5,60}$ 1.71, P<0.001). The choice of refugia habitats by *B. bufo* did not differ with their origin, sex or between 1994 and 1995 (P_{all}>0.05). The log-ratio differences for each habitat were therefore pooled across years and sexes for the pair-wise analysis. The results of the pair-wise analysis are shown in table 3.4.

Table 3.4

Compositional Analysis Summary - Habitat Choice Of *B. bufo* In Refugia 1994 And 1995

All Animals

	HABITAT						
	Hedge	Edge	Wood	Arable	Rough	Pasture	RANK
Hedge		-0.59	**-2.69**	1.28	**-3.61**	1.35	2
Edge	0.59		-1.87	1.87	**-3.21**	**1.99**	3
Wood	**2.69**	1.87		**4.25**	-0.82	**4.24**	4
Arable	-1.28	-1.87	**-4.25**		**-4.91**	0.29	1
Rough	**3.61**	**3.21**	0.82	**4.91**		**4.87**	5
Pasture	-1.35	**-1.99**	**-4.24**	-0.29	**-4.87**		0

Significant differences are shown in bold. The rank is the number of positive values and shows the order of preference from most preferred with a rank of 5 to least preferred with a rank of zero. Data are mean of the pair-wise comparisons of the log-ratio differences between the use and availability of each habitat. In all cases edge was chosen as the denominator in the calculation of log-ratios.

The order of preference from most preferred to least preferred for refugium habitats was:

Rough grassland > Woodland > Edge > Hedge > Arable > Improved pasture.

Rough grassland had the highest preference rank and was significantly preferred to all other habitats except woodland. Woodland was significantly preferred to hedge, arable and improved pasture; edge was significantly preferred to improved pasture.

3.5.3 Habitat Use By Active Animals 1994 And 1995

The habitat use by male and female *B. bufo* in 1994 and 1995 are given in Figures 3.6(a,b,c,d) respectively. All *B. bufo* used rough grassland and woodland more than the other 4 habitats. For females in 1994 and for all *B. bufo* tracked in 1995 only woodland and rough grassland had percentage fixes of greater than 10%. Males in 1994 showed greater than 10% of fixes in edge, hedge, arable and pasture in at least one month. For all *B. bufo* in both years there was an increase in the use of woodland from May to August and a corresponding decrease in the use of rough grassland.

Habitat preference by active *B. bufo* differed significantly from random (Hotellings $T^2_{5,60}.00$, $P<0.001$). Foreign and local *B. bufo* did not differ significantly in their habitat use and were pooled for all further analyses ($P>0.05$). Habitat choice differed significantly both between years (Hotellings $T^2_{5,53}0.47$, $P<0.005$) and between sexes (Hotellings $T^2_{5,53}=0.23$, $P<0.05$). There were no significant interactions between any of the three independent variables. The log-ratio differences for males and females in each of the two years were kept separate for the pair-wise analysis. The results of the pair-wise analysis are shown in table 3.5

Mean pairwise comparisons of habitat use by active males and habitat availability were not significantly different from zero in 1994 therefore males in this year were using habitats in proportion to their availability. The order of preference from most preferred to least preferred for all other active *B. bufo* was:

(FEMALES 1994)
Rough grassland>Woodland>Edge>Hedge>Arable>Improved pasture

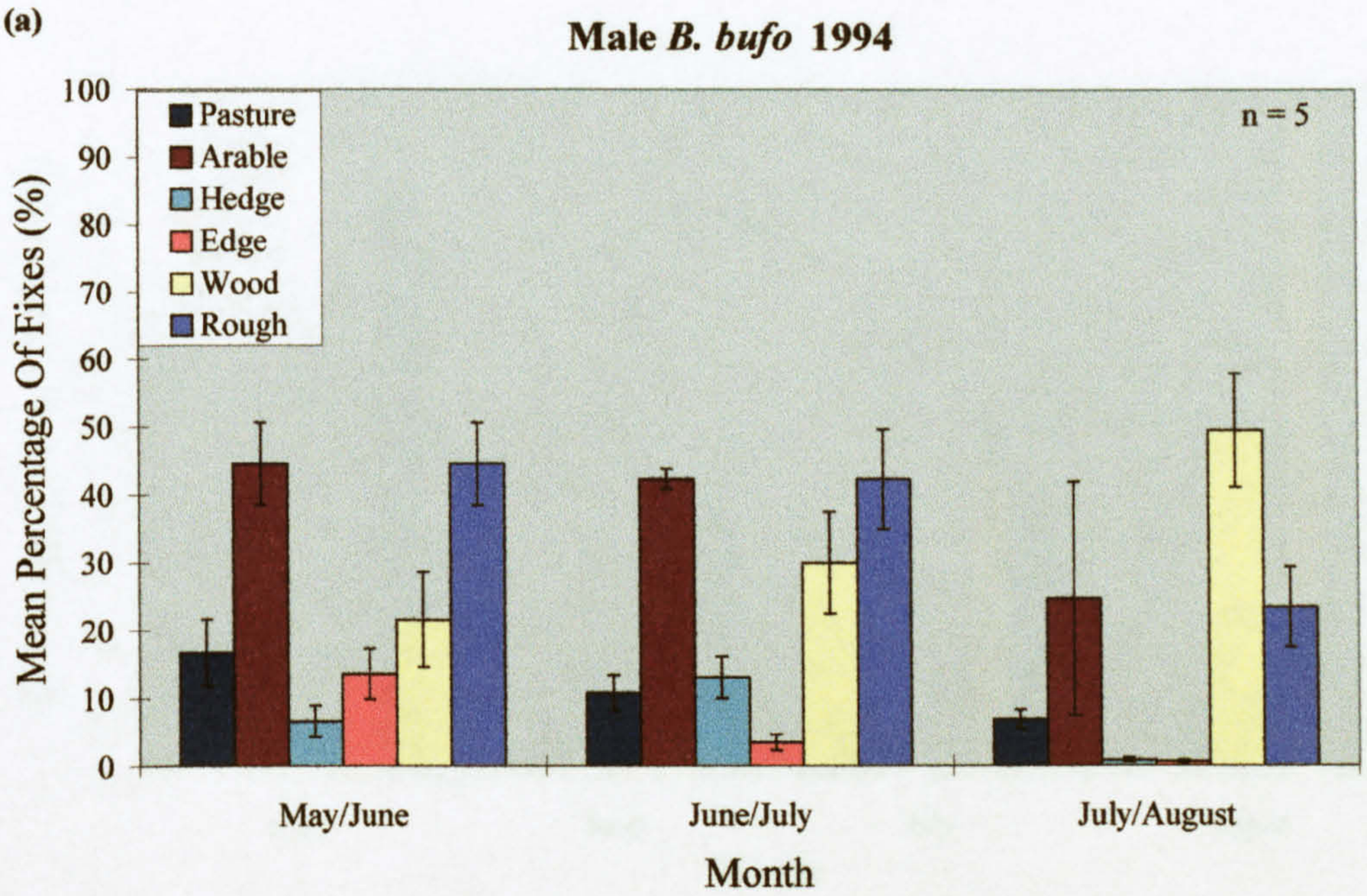
(MALES 1995)
Rough grassland>Woodland>Edge>Hedge>Arable>Improved pasture

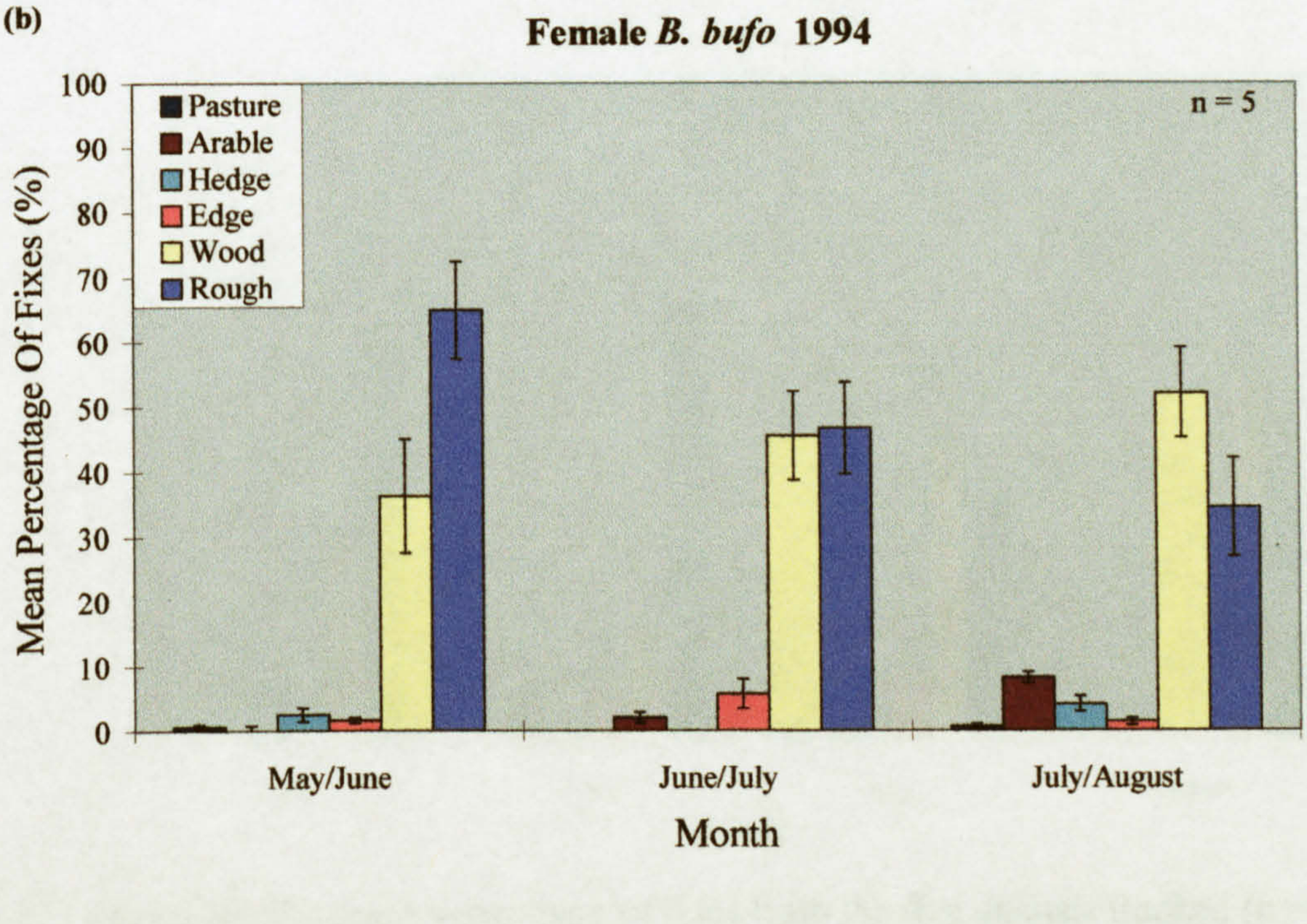
(FEMALES 1995)
Rough grassland>Woodland>Edge>Hedge>Arable>Improved pasture

Females in 1994 significantly preferred rough grassland to all other habitats except woodland. Woodland was significantly preferred to both arable and improved pasture habitats by the 1994 females. In 1995 the males significantly preferred both the rough grassland and woodland to all other habitats.

Figure 3.6

Habitat Use By Radio-tracked *B. bufo* In The Active Phase



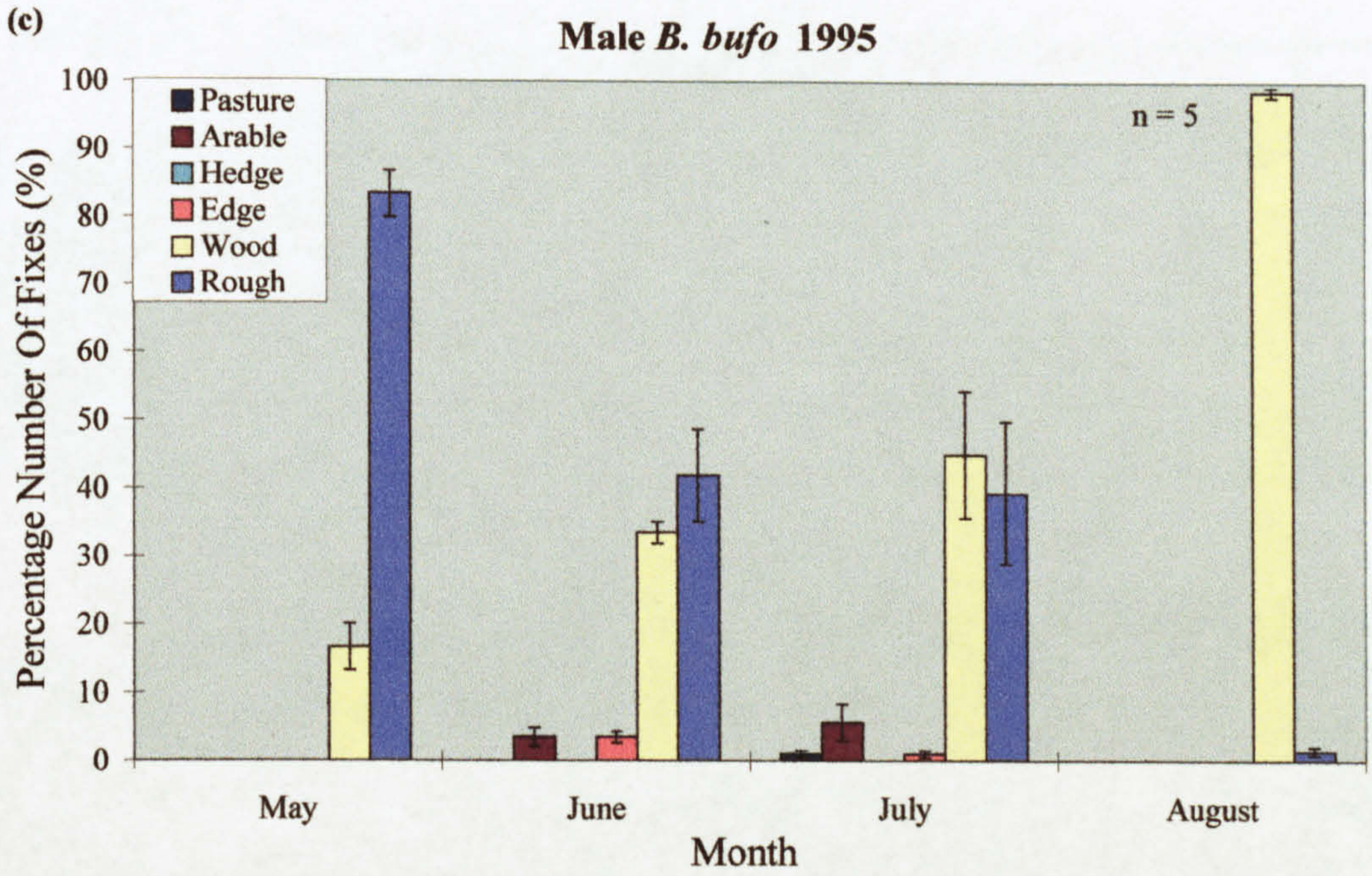


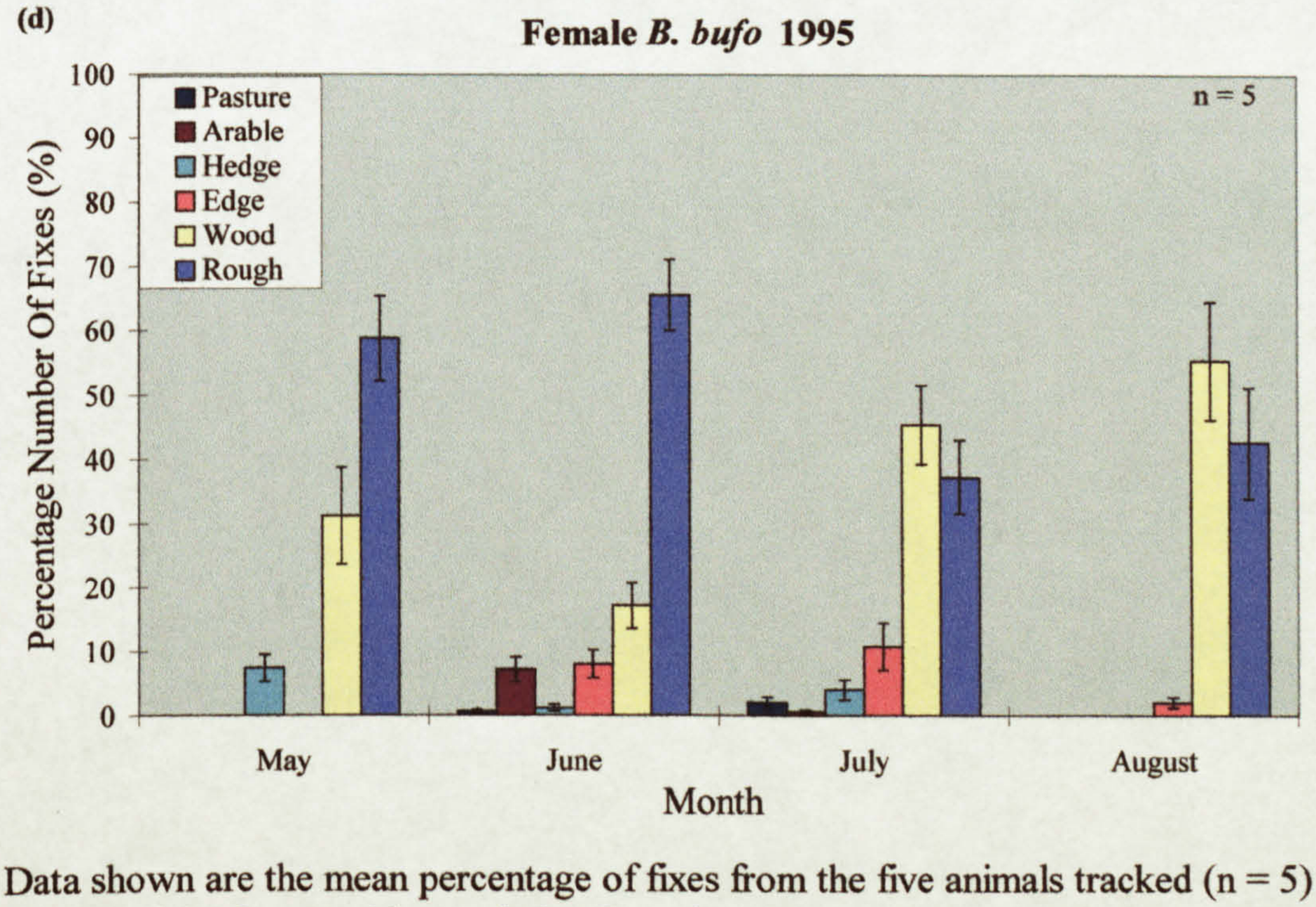
Tracking Periods
Ma/Ju = 6th May to 6th June
Ju/Jy = 7th June to 16th July
Jy/ Au = 18th July to 24th August

Data shown are the mean percentage of fixes from the five toads tracked (n = 5) in each of the three tracking periods. Error bars are +/- one standard error of the mean.

Figure 3.6 (cont.)

Habitat Use By Radio-tracked *B. bufo* In The Active Phase





Data shown are the mean percentage of fixes from the five animals tracked (n = 5) in each of the four tracking periods. Error bars are +/- one standard error of the mean.

Table 3.5

Compositional Analysis Summary - Habitat Choice Of Active *B. bufo* In 1994 And 1995

Males 1994

	HABITAT						
	Hedge	Edge	Wood	Arable	Rough	Pasture	RANK
Hedge		-0.08	1.12	0.65	-2.23	-0.79	2
Edge	0.08		0.16	0.78	-2.17	-0.70	3
Wood	-1.12	-0.16		0.57	-2.33	-0.87	1
Arable	-0.65	-0.78	-0.57		-2.89	-1.44	0
Rough	2.23	2.17	2.33	2.89		1.46	5
Pasture	0.79	0.70	0.87	1.44	-1.46		4

Females 1994

	HABITAT						
	Hedge	Edge	Wood	Arable	Rough	Pasture	RANK
Hedge		-0.55	**-3.55**	1.57	**-3.69**	1.83	2
Edge	0.55		-0.96	2.12	**-3.15**	2.60	3
Wood	**3.55**	0.96		**5.17**	-0.17	**5.68**	4
Arable	-1.57	-2.12	**-5.17**		**-5.27**	0.48	1
Rough	**3.69**	**3.15**	0.17	**5.27**		**5.74**	5
Pasture	-1.83	-2.60	**-5.68**	-0.48	**-5.74**		0

Males 1995

	HABITAT						
	Hedge	Edge	Wood	Arable	Rough	Pasture	RANK
Hedge		-1.32	**-5.04**	0.62	**-5.17**	**1.39**	2
Edge	1.32		**-3.72**	1.95	**-3.85**	**2.70**	3
Wood	**5.04**	**3.72**		**5.66**	-0.60	**6.42**	4
Arable	-0.62	-1.95	**-5.66**		**-5.80**	0.75	1
Rough	**5.17**	**3.85**	0.60	**5.80**		**6.55**	5
Pasture	**-1.39**	**-2.70**	**-6.42**	-0.75	**-6.55**		0

Females 1995

	HABITAT						
	Hedge	Edge	Wood	Arable	Rough	Pasture	RANK
Hedge		-0.44	**-3.15**	2.04	**-3.41**	**2.58**	2
Edge	0.44		-2.70	2.49	**-3.56**	**3.02**	3
Wood	**3.15**	2.70		**5.25**	-0.33	**5.34**	4
Arable	-2.04	-2.49	**-5.25**		**-5.49**	1.10	1
Rough	**3.41**	**3.56**	0.33	**5.49**		**5.50**	5
Pasture	**-2.58**	**-3.02**	**-5.34**	-1.10	**-5.50**		0

Significant differences are shown in bold. The rank is the number of positive values and shows the order of preference from most preferred with a rank of 5 to least preferred with a rank of zero. Data are means of the pair-wise comparisons of the log-ratio differences between the use and availability of each habitat. In all cases edge was chosen as the denominator in the calculation of log-ratios.

Hedge and edge were both significantly preferred to improved pasture by the 1995 males. The 1995 females significantly preferred rough grassland to all other habitats except woodland. Woodland was significantly preferred to improved pasture and arable by the 1995 females and edge and hedge were also significantly preferred to improved pasture.

3.5.4 Mass Change And Mortality Of Radio-tracked Animals

The mean mass changes of radio tracked *B. bufo* are show in Figure 3.7. The final mass was significantly greater than the initial mass ($T_{1,82}$=3.35, P<0.01) so *B. bufo* gained mass overall. Figure 3.7 shows the large standard errors of the mean mass changes. The mean mass changes did not differ significantly between sexes but did differ significantly between years ($F_{2,82}$=2.75, P<0.05). Females in 1995 showed a mean mass loss of 0.1g. Males in 1995 had a mean mass gain of 1.1g. The mass change, number of days tagged and fate of tracked *B. bufo* are shown in Tables 3.6a and 3.6b.

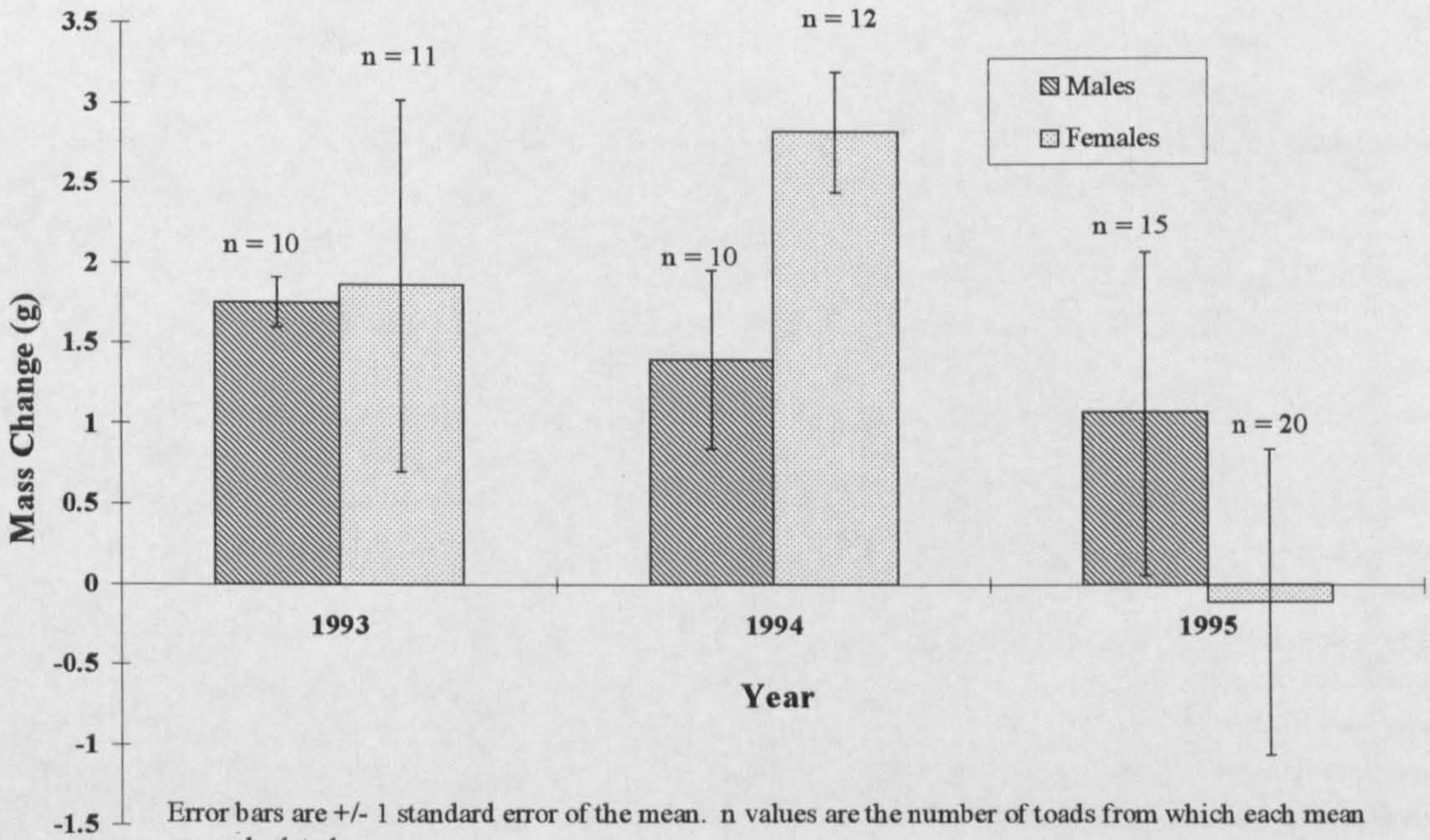
Five of the 30 animals (16.6%) were predated in 1994 and only two out of 40 in 1995 (5%). In 1995 in July and August 10 animals died probably due to dehydration, seven of these were males. Five males died before adequate numbers of radio-tracking fixes could be obtained and are marked with a * in table 3.6. These males were excluded from analysis on habitat utilisation. The mean number of days tagged was 19.5 days for males and 21.2 days for females, in 1994. The mean number of days tagged in 1995 was 12.2 for males, 14.4 for females. If the five dehydrated males are excluded from the calculation then the mean number of days tagged for males in 1995 was 16.3 days.

3.5.5 Behaviour Of *B. bufo*

The mean percentage occurrence of each of the three behavioural categories are shown in Figure 3.8. *B. bufo* showed 'sitting' behaviour in a mean of greater than 80% of observations. Feeding was rarely seen (mean <5% of observations). Males moved significantly more than the females in 1994 ($\chi^2_{1,30}$= 5.30,P<0.05) but not in 1995 ($\chi^2_{1,35}$= 0.01,P>0.05).

Figure 3.7

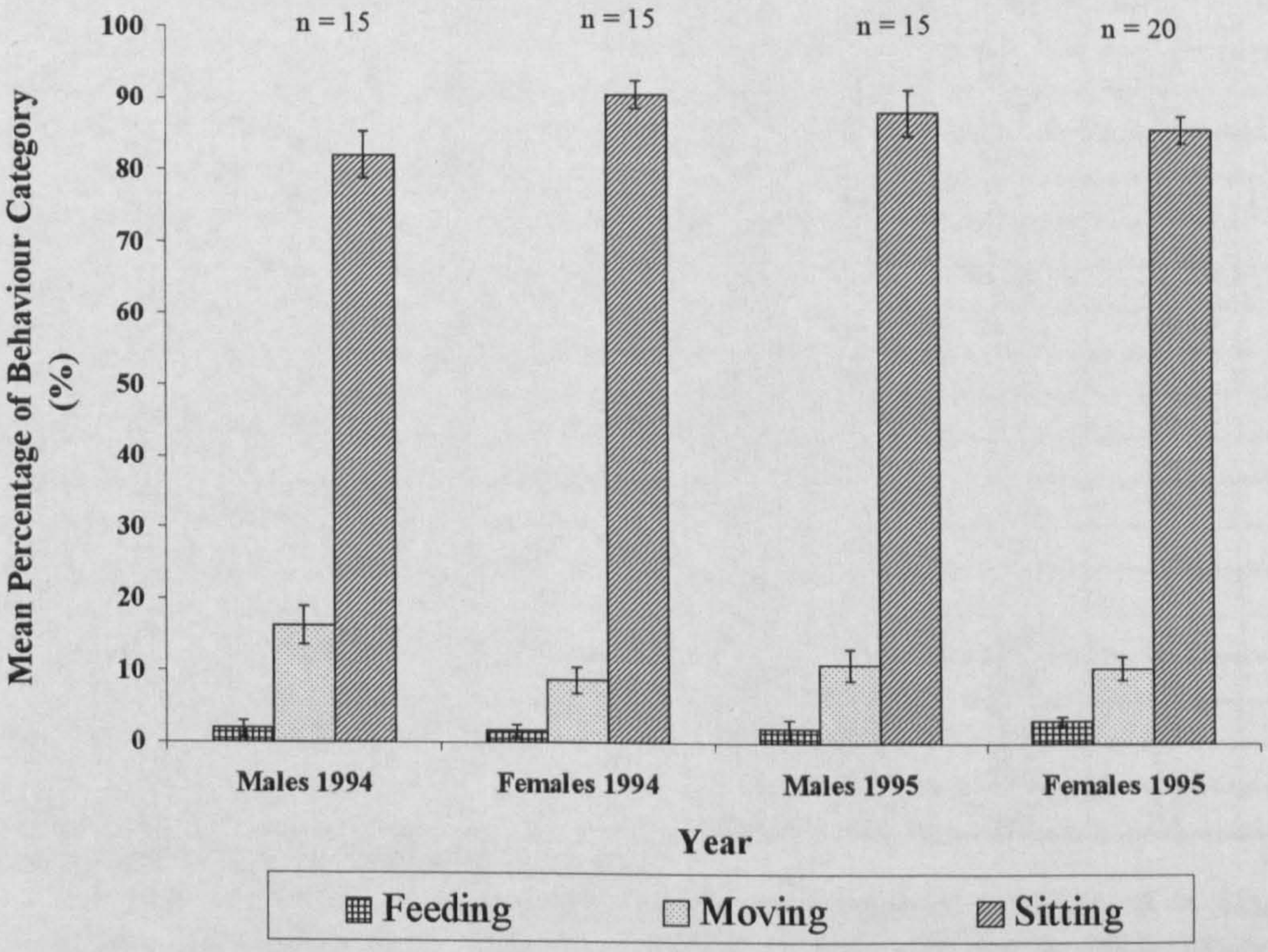
Mean Mass Change of Radio-tracked *B. bufo*



Error bars are +/- 1 standard error of the mean. n values are the number of toads from which each mean was calculated.

Figure 3.8

Behaviour of Radio-tracked *B. bufo*



Behaviour was recorded in one of the three categories each time a *B. bufo* was located. The data for each *B. bufo* were converted to percentages. Data shown are the mean of all *B. bufo* +/- one standard error of the mean. n values are the number of *B. bufo* tracked.

Table 3.6

Mass Change And Mortality Of Radio-tracked *B. bufo* With Ingested Tags

(a) 1994

MALES					FEMALES				
B. bufo	Initial Mass (g)	Mass Change (g)	Number Days Tagged	Fate	*B. bufo*	Initial Mass (g)	Mass Change (g)	Number Days Tagged	Fate
AM1	26.2	-	10	P	AF1	29.6	2.1	32	R
AM2	28.4	-	10	R	AF2	32.9	0.6	32	P
AM3	32.5	1.6	16	P	AF3	31.0	3.4	32	P
AM4	26.4	**-2.0**	33	R	AF4	44.6	2.4	11	R
AM5	29.1	1.9	32	R	AF5	39.9	4.7	42	F
AM6	32.0	2.5	31	E	AF6	34.2	4.5	11	R
AM7	25.8	**-0.6**	33	E	AF7	34.4	3.1	8	R
AM8	26.9	-	11	R	AF8	26.1	0.6	21	E
AM9	29.7	0.9	17	R	AF9	26.4	4.0	21	P
AM10	25.9	1.5	20	F	AF10	40.6	3.0	43	E
AM11	26.3	1.3	21	R	AF11	33.0	2.6	14	R
AM12	26.3	-	5	R	AF12	37.3	-	12	R
AM13	23.9	2.3	23	R	AF13	31.1	-	5	R
AM14	32.8	4.6	23	R	AF14	26.5	-	23	R
AM15	25.9	-	7	R	AF15	45.0	3.0	11	E

(b) 1995

MALES					FEMALES				
B. bufo	Initial Mass (g)	Mass Change (g)	Number Days Tagged	Fate	*B. bufo*	Initial Mass (g)	Mass Change (g)	Number Days Tagged	Fate
AM1	26.3	6.2	23	R	AF1	43.7	2.3	17	E
AM2	32.2	10.1	16	R	AF2	45.8	4.2	17	E
AM3	27.2	1.9	20	P	AF3	36.1	1.7	10	P
AM4	21.4	1.6	20	R	AF4	42.3	3.0	10	R
AM5	21.4	**-0.2**	10	R	AF5	48.2	**-3.8**	21	E
AM6	25.8	3.2	20	R	AF6	38.4	2.1	10	R
AM7	27.8	0.9	17	R	AF7	48.9	7.9	17	E
AM8	25.9	1.3	17	R	AF8	44.5	**-4.9**	17	E
AM9	25.2	1.2	17	E	AF9	51.5	4.4	17	E
AM10	27.7	0.6	17	R	AF10	46.6	**-1.1**	17	E
AM11	38.0	1.7	16	R	AF11	42.4	4.0	16	E
AM12	25.8	0.3	16	R	AF12	36.9	0.3	16	R
AM13	25.1	**-2.1**	16	R	AF13	29.6	**-1.4**	16	R
AM14	26.5	-	3*	D	AF14	32.6	0.9	16	R
AM15	30.2	-	3*	D	AF15	32.1	2.1	16	E
AM16	28.3	**-3.8**	10	D	AF16	41.7	**-0.8**	11	D
AM17	29.4	-	2*	D	AF17	43.3	**-8.1**	11	E
AM18	30.1	**-6.9**	10	D	AF18	37.5	**-2.3**	11	E
AM19	28.3	-	2*	D	AF19	28.7	**-4.5**	11	D
AM20	26.2	-	4*	D	AF20	36.5	**-8.2**	11	D

Negative mass changes are highlighted in bold.

P = Predated. Corpse of *B. bufo* found showing clear signs of predation. R = Regurgitated. Transmitters regurgitated by *B. bufo*. E = Ended. *B. bufo* collected to allow removal of old transmitters.

F/UK = Failed/Unknown. Neither *B. bufo* nor transmitter located. D = Died. Animal showed signs of dehydration before death and with no other cause of death evident.

3.6 DISCUSSION

3.6.1 Mass Change And Mortality Of Radio-tracked Animals

The size threshold for radio-tracked *B. bufo* was set at 25g in chapter 2 as this produced a 10% transmitter mass to body mass ratio. By 1995 transmitter modifications by the manufacturers (Biotrack, Wareham, Dorset, UK) had reduced the mass of the transmitters to a mean of 2.0g allowing smaller (<25 g) *B. bufo* to be tagged. Radio-tracked *B. bufo* had significant ($P<0.05$) mass gains overall, and appeared to show normal feeding behaviour.

The transmitters may have increased the stress of *B. bufo* particularly in 1995 when weather conditions were already subjecting animals to desiccation stress. Dehydrated *B. bufo* from the local population were observed in July and August in the fields surrounding the study site. Corpses observed in the surrounding area showed no signs of predation or parasitism. This suggests that the death of *B. bufo* within the radio-tracking area was primarily a weather-related rather than transmitter- related mortality. 10% of *B. bufo* were predated with a higher percentage predation in 1994 than in 1995. Predators were mostly birds with clear peck marks often on still living animals. The higher predation in 1994 may have resulted from the increased movements (See Figure 3.8) and higher use of open habitats in 1994 (see Figure 3.6) making *B. bufo* more visible to predators. The mortality increased in 1995 due to the dehydration of 10 animals. It is not possible to determine whether tagged animals showed a larger percentage mortality due to predation or dehydration compared with untagged animals. Predation of tagged animals is discussed further in section 6.11.

In 1993 and 1994 *B. bufo* gained significantly more mass than *B. bufo* in 1995; females in 1995 showed a mean mass loss. The differences in mass changes between the three years are likely to reflect the different weather conditions and therefore desiccation states of *B. bufo*. *B. bufo* were hydrated before release and before initial weighing. Therefore mass loss are most likely to reflect the dehydration of *B. bufo* in the field. The relationship between microclimate and mass changes are discussed in chapters 6 and 8.

A total of 34 out of 70 *B. bufo* tracked in 1994 and 1995 regurgitated their transmitters. The minimum number of fixes were obtained for all of these *B. bufo* prior to regurgitation. The mean tracking times were comparatively short (12.2 days males 1994) and in longer term studies the loss of transmitters due to regurgitation may result in loss of valuable data.

3.6.2 Refugia Preference

B. bufo demonstrated a clear habitat preference for refugia in woodlands and rough grasslands (Figure 3.5). These habitats were chosen almost exclusively by both males and females. The refugia habitats were significantly more diverse (have higher H' values) than random ($P<0.05$) but the causative factors in their selection are more likely to be the provision of suitable microclimate (see chapters 6 and 8).

Refugia habitat selection differed significantly ($P<0.05$) from habitat selection by active *B. bufo*. On limited occasions *B. bufo* appeared to move from the refugia sites into feeding grounds and then return to refugia sites. Van Gelder *et al* (1986a) studying the migrating movements of *B. bufo* noted that when temperature and humidity decrease *B. bufo* moved into refugia. This anecdotal evidence is supported by the current data, as active *B. bufo* were able to use more open habitats on a limited number of occasions but only used the more closed habitats for refugia. The interaction between refugia habitat choice and microclimate variables and the implications of refugia habitat preference for the survival of *B. bufo* are discussed in chapters 6 and 8.

3.6.3 Habitat Preference And Behaviour Of Active *B. bufo*

The most preferred habitats of *B. bufo* during the active phase were the rough grassland and woodland habitats. The percentage use of the other habitats was much greater in active *B. bufo* than by those in refugia (3.6.2). Local and foreign *B. bufo* were from populations distant enough to ensure little genetic mixing and yet no significant differences in habitat preferences were found. This implies habitat preference is constant under the same conditions for *B. bufo* of the same sex from the same geographical region.

B. bufo habitat preferences differed significantly both between years ($P<0.05$) and between sexes ($P<0.05$) due to the behaviour of the male *B. bufo* in 1994. Males in 1994 showed a tendency to move into non-refugia habitats such as arable and edge but they used these habitats according to their availability showing no overall habitat preference. Males in 1994 moved significantly more than females in the same year. This resulted from forays into the more open habitats when *B. bufo* were active, followed by return to the refugia habitats; woodland and rough grassland. The differences in behaviour of males in 1994 compared with 1995 are linked to the differences in the prevailing weather conditions. 1994 was a cool wet summer and 1995 was a hot dry summer. The link between microclimate and habitat selection is discussed in chapter 6. Possible reasons for the differences in male and female behaviour are discussed in chapter 8.

The enclosure in the current study provided a controlled environment where habitats could be managed and manipulated. Given the choice of habitats within the enclosure female *B. bufo* in 1994 and all *B. bufo* in 1995 demonstrated a preference for woodland and rough grassland. The results must be considered within the limitations of a single site enclosure study. Enclosures are of fixed size and this limits the numbers of animals that can be studied simultaneously due to density effects. In a ten year study Reading and Clarke (1995) suggested interspecific competition for food caused low body conditions in *B. bufo* at high densities. At high densities it is possible that habitat choice of a radio-tracked animal could be influenced by density, with the weakest competitors being pushed out into sub-optimal/non-preferred habitats (White and Garrott 1990).

The density of radio-tracked *B. bufo* in the current study was 100 animals per hectare (10 animals in the 0.1 Hectare enclosure). If all animals in the current study preferred the smallest main habitat (woodland) the maximum density would be 588 *B. bufo* per hectare. Gittins *et al* (1980) estimated a density of 23 breeding *B. bufo* per hectare whereas Frazer (1966) only estimated one *B. bufo* per hectare. Compared to these studies the density in the radio-tracking enclosure is high. However both of these studies assumed that *B. bufo* were evenly distributed throughout all habitats around a breeding site. Latham (1997) used a combination of fences and year round pitfall

trapping to calculate the density of *B. bufo* in different habitats around a single breeding pond in Leicestershire. Density varied with between habitats with 56 *B. bufo* per hectare in wheat arable habitats 200 *B. bufo* per hectare in mixed deciduous woodland to a staggering 1200 *B. bufo* per hectare in the understorey of a poplar plantation (Latham 1997). The Latham (1997) study was conducted in the same area (N.W. Leicestershire) as the current study and therefore the density of *B. bufo* within the enclosure was within those observed in the wild.

The extrapolation of results from single site behavioural studies to general comments on species behaviour should be cautious. Every individual site will vary in abiotic and biotic factors. For example the plant species, edaphic features and the degree of slope may vary between sites. Any or all of these features may affect the behaviour of a species and therefore the results obtained.

Despite these caveats the preference for woodland shown in the current enclosure study has also been noted in other field-based studies (Beebee 1977b, 1979, 1981, Oldham and Swan 1991, Denton and Beebee 1994). Beebee (1977b, 1979, 1981) compared the terrestrial habitats surrounding amphibian breeding ponds with the habitats around non-breeding ponds. He observed that a woodland component is required in the terrestrial habitat for the presence of *B. bufo* at breeding sites. Denton and Beebee (1994) used a combination of night searches, radio-tracking and vivarium experiments to monitor the habitat preferences of *B. bufo* and *B. calamita*. They found that *B. bufo* were associated with complex habitat types such as scrub and lost condition when transferred to open habitats.

Oldham and Swan (1991) showed that woodland and scrub supported higher densities of *B. bufo* in better condition compared with arable and pasture habitats. The importance of woodland and scrub are clearly evident from these studies and agree with the findings of the present study. The importance of long/rank grassland was not however, highlighted in any of these studies. The Denton and Beebee study (1994) was conducted on sand dune and heath systems with no equivalent long rank grassland habitats available. The Oldham and Swan (1991) study contained areas of rank

grassland with trees. These areas were classified as scrub and were the highest quality habitats, agreeing with the findings of the current study.

Pasture and arable were used significantly less than almost all other habitats in the current enclosure study. Beebee (1981) did not find *B. bufo* breeding in any pond in his agricultural lowland survey and Oldham and Swan (1991) found *B. bufo* from arable and pasture sites were in relatively poor condition.

Strijbosch (1980) studied habitat use by nine amphibian species including *B. bufo* using a combination of night searches and pitfall trapping. Strijbosch found *B. bufo* occurred more frequently in open habitats than in woodland, which contradicts the data from other studies. Re-examination of his data suggests that this difference may be an artefact of differences in the way data were collected, pooled and analysed. Strijbosch pooled hay meadows with pastures in the open habitat category. This would be the equivalent of pooling the most preferred habitat (long grassland) and least preferred habitat (improved pasture) in the current study. The data for his statistical analysis were based on direct observations with no account made of sample bias due to ease of viewing in different habitat types. By definition *B. bufo* would be easier to see in the open compared with the closed habitats. The differences between the Strijbosch and current study may also be explained by differences in overall climatic and edaphic conditions. The Strijbosch study was conducted on a dry heath/dune system in the Netherlands.

B. bufo may be able to adapt their use of habitat to the prevailing weather conditions, demonstrating a type of behavioural thermo/moisture regulation. The cost of moisture loss and increased predation risk in more open habitats is probably being balanced by the benefit of enhanced provision of food. For example the increased use of woodland and decreased use of rough grassland from May - August may reflect adaptation to changing microclimatic conditions. This movement from rough grassland to woodland was not tested statistically as the number of animals tracked per tracking period was low (five males and five females). A discussion of the interactions between prey provision, prey selection in different habitats is given in chapters 4 and 5. The results of the current enclosure study are compared with population and habitat data from 26

sites in chapter 7. Microclimate in different habitats are discussed in chapter 6 and the interactions between microclimate, prey selection, prey availability and habitat preference are discussed in chapter 8. The implications of habitat preference for *B. bufo* survival are discussed in chapter 8.

3.7 CONCLUSIONS.

- Habitat selection by *B. bufo* in passive and active phases differed significantly.

- *B. bufo* demonstrated significant preferences for woodland and rough grassland habitats by comparison with arable and pasture habitats.

- Habitat preferences differed significantly between sexes and years due to increased use of arable, edge and pasture habitats by males in 1994.

4: PREY AVAILABILITY

4.1 INTRODUCTION

Food availability in different habitats influences the fecundity and growth rate of *B. bufo* (Gittins 1987; Swan 1986). *B. bufo* on a restricted diet show reduced body fat and a decline in spermatogenic activity (Guha *et al* 1980). Food availability is likely to influence the reproductive state of an animal and influence the carrying capacity of the terrestrial habitat. Sinsch (1988) suggested that size of *B. bufo* home ranges depended on habitat linked factors such as prey availability. In order to determine the quality of habitats and the mechanism of habitat selection, I studied the prey available to and prey selected by *B. bufo* during April-September 1993-1995. This chapter will concentrate on the prey availability in different habitats. Chapter 5 compares the prey selected by *B. bufo* with prey availability.

4.1.1 Invertebrate Sampling

Two of the most commonly selected methods of assessing invertebrate abundance are pitfall trapping and suction sampling. Pitfall traps offer a potential method of invertebrate sampling which may imitate the sit-and-wait strategy used by many adult amphibians (Toft 1981) which are discussed further in chapter 5. The problems involved in the use of pitfall traps have been well documented by Southwood (1978). Pitfall trap catches have been shown to differ with variations in the following features:

1) Climatic conditions (Greenslade 1961)
2) Vegetation immediately surrounding the trap (Baars 1979)
3) Surface irregularities (Obrtel 1971)
4) Species, behaviour and sex of invertebrate (Grum 1966, Luff 1978, Halsall and Wratten 1988, Benest 1989a, Topping and Sunderland 1992).
5) Shape and material of the trap (Greenslade and Greenslade 1971, Waage 1985, Benest 1989b).
6) Trap diameter (Baars 1979)
7) Killing agent used (Luff 1968, 1975, Greenslade and Greenslade 1971).
8) Cover of trap (Baars 1979)
9) Digging in effects (Greenslade 1973).

Errors from the aforementioned problems can be reduced by experimental design. A killing agent consisting of water with a drop of "photoflow" to break the surface tension (Swan 1986) prevents the problems involved with the use of Formalin (Luff 1968) and methylated spirits (Greenslade and Greenslade 1971). A wooden cover, prevents problems caused by changes in the microclimate around the pitfall (Baars 1979). In habitat comparisons, standardisation of trap design between habitats should overcome problems five and six (listed above). Problems of digging-in' noted by Greenslade (1973) can be minimised by taking samples over a long period (April-September). Long term sampling is more representative of invertebrate communities present (Benest 1989b) and the relationship between year round sample numbers and actual species density was close to linear (Baars 1979).

Cornish (1992) recommended a second method of invertebrate sampling to reinforce the pitfall catch data. Suction traps have been used successfully to sample a wide range of invertebrates including Coleopterans (Arnold *et al* 1973) and Fleahoppers (Richmond and Graham 1969). The efficiency of the suction samplers is chiefly determined by the invertebrate being sampled (Henderson and Whitaker 1977) so is consistent between different habitats. Suction sampling therefore provides a method of reinforcing pitfall trap data to try to reduce the errors resulting from pitfall trap avoidance by different invertebrate behaviours.

4.2 APPROACH AND AIMS

The aim of this chapter is to compare the availability and distribution of invertebrate prey in different habitats. Suction sampling and pitfall trapping were used to sample the prey availability in the radio-tracking area (described in chapter 2). To ameliorate any potential sampling errors the two methods were used in each habitat and invertebrates were sampled for the duration of the *B. bufo* non-breeding activity. Since *B. bufo* are mainly nocturnal/crepuscular the diurnal fluctuations in prey availability were also assessed by closing selected traps during the day. Availability of prey in different habitats was compared in terms of dry mass and total numbers (abundance) of trapped invertebrates. The distribution of invertebrates in different habitats was compared in terms of percentage abundance of different taxa and different size categories of

invertebrates. Dry mass, total numbers, distribution of taxa and size categories of invertebrates in nocturnally opened traps were compared with those found in traps open for 24 hours.

4.3 MATERIALS AND METHODS

The investigation was conducted in the radio-tracking area which is described in chapter 3. Only the main habitat areas were sampled i.e.: improved pasture, rough grassland, woodland and arable. The main habitats were sampled to allow invertebrate catch to be compared with microclimate data which was only measured in the main habitats (chapter 6). The hedge and edge are linear land features and are by definition narrow habitats (<4m wide). Pitfall traps sample invertebrates from wide circumferences around the trap (Adis 1979). Pitfall traps in narrow habitats will catch invertebrates from all habitats adjacent to the sampled habitat (Luff 1975).

4.3.1 Pitfall Trapping

On 14 April 1993 five pitfall traps were dug into each of the four main radio-tracking habitats. Pitfall traps sample from Obrtel (1971) states that ten pitfalls will catch the dominant Carabidae within a habitat, however Cornish (1992) found no significant difference in the frequency size class distributions from five and ten pitfalls. The maximum area of a single habitat monitored in the current study was approximately 200m^2 (improved pasture in 1993) and five pitfalls were deemed sufficient for this area. The pitfalls were arranged in a cross pattern spaced at least five metres apart to trap invertebrates from the maximum area.

Pitfall traps were made from a two litre plastic bottle with both base and upper quarter removed to form a cone. Plastic cups (diameter 65mm) by DRG plastics Ltd were placed flush with the ground surface inside these outer containers. This design was cheap and allowed frequent and easy removal of the inner trap without causing repeated disturbance of the ground, which can alter the pitfall catch (Greenslade 1973). A 6cm depth of water with a drop of wetting agent (photoflow) to break the surface tension was used in each trap. Wooden covers 400cm^2 were supported 100mm above

the ground. The cover protected the trap from flooding by rainfall and stopped blockage by debris.

In 1993 pitfall traps were emptied weekly from April 26th to August 24th. The pitfall contents were sieved using a fine mesh sieve (mesh size 0.3mm) and the retained material preserved in Formalin (10%). The same procedure was used to sample invertebrates from May 1st to August 28th in 1994 and from May 1st to August 25th in 1995 except that pitfalls were emptied every two weeks in 1994 and 1995 to reduce the number of man hours in identification of invertebrates. To preserve the pitfall catch for this longer catch period 50% ethane diol, 30% distilled water and 20% ethanol were used as the pitfall solution.

To determine if nocturnal distribution of taxa differed from diel distributions two extra pitfalls were added to each habitat in May 1994. These were covered using a foil cover. The traps were opened at dusk and closed at dawn for five days in each month from May - August in 1994 and 1995. Data from the five traps in each habitat open 24 hours per day were termed the diel catch and data from the covered traps were termed the "nocturnal" pitfall catch.

4.3.2 Suction Trapping

The enclosure was suction trapped at night on three occasions in 1993 the 28th May, 1st June and 10th July. In 1994 suction sampling occurred on the 10th July and in 1995 on the 20th of June. The Burkhard suction trap used, consisted of a portable vacuum pump powered by a two-stroke petrol engine with a narrow suction hose. A small plastic bucket with the base removed (diameter 220mm) was placed on the ground to enclose all vegetation within its' surface and this area was sampled for two minutes. The invertebrates and debris collected from three x two minute collection periods were pooled as one sample, there were six samples per habitat. Any large invertebrates were removed on an initial sorting. The samples were then placed into Tulgren Funnels and left for 48 hours. The samples were examined for any invertebrates missed in the initial sorting. The invertebrates from the initial sorting and the Tulgren funnel tubes were identified.

4.3.3 Invertebrate Identification And Measurement

Invertebrate samples were examined using a binocular microscope (magnification range x30 and x10). Non-arthropod invertebrates were classified to Phyla with 2 notable exceptions. Annelida were separated into Oligochaeta and other Annelida, the phylum Mollusca was separated into the subclass Pulmonata and other Mollusca. Insecta, Crustacea and Myriapoda were identified to order except Coleopterans which were identified to family. Guides used in invertebrate identification were Unwin (1981, 1984), Tilling (1987), Jones-Walters (1989), Wright (1990), Hopkin (1991) and Chinery (1986). Maximum length of all invertebrates was recorded (from the posterior tip of the abdomen to the anterior tip of the head) in mm (+/- 0.25mm). The invertebrates were sorted into size classes shown in Table 4.1. Abdominal swelling occurred in many of the larger Coleopteran species caused by absorption of the pitfall trap solution. The length of larger beetles was therefore taken from to the anterior tip of the head to the posterior tip of the elytra. The smallest size of invertebrate recorded was 0.3mm as this was the mesh size of the sieve used to separate invertebrates from the trapping solution. The numbers of each taxon and size class were recorded by direct counting.

Table 4.1

Size Classes of Invertebrates

Size Class	1	2	3	4	5	6	7	8	9	10	11
Invertebrate Size (mm)	0.3 - 1.9	2.0 - 3.9	4.0 - 5.9	6.0 - 7.9	8.0 - 9.9	10.0- 11.9	12.0 - 13.9	14.0 - 15.9	16.0 - 17.9	18.0 - 19.9	>/= 20.0

Diel pitfall catches from consecutive sampling dates were pooled to give monthly samples: May, June, July and August. Pooling allowed comparison between years and with radio-racking and microclimate data (Chapter 3 and Chapter 6). After identification and measurement, samples from 1994 and 1995 were dried to constant mass and total dry mass per pitfall, per month was recorded. During August 1995 more intensive analysis of food availability aimed to determine the contribution of

different taxa to the total pitfall dry mass. The catches from August 1995 were identified, counted, measured and then the dry mass of each taxon per pitfall was recorded. Dry mass per taxon was determined in only one month to reduce the number of man hours on analysis of each pitfall. No dry mass was recorded form nocturnal traps as these were open for a only 5 days a month and therefore data was not comparable with that from diel traps which were open all month.

4.3.4 Microclimate Measurement

Microclimate data were collected at ground level in each of the four main habitat enclosures; arable, improved pasture, rough grassland and woodland. One pair of "*Tinytalk*" temperature and humidity data loggers was placed at ground level in each of the four main habitats. Collection of microclimate data is described more fully in chapter 6.

4.3.5 Statistical Analysis Of Pitfall Trap Data

The monthly percentage distribution of taxa in each pitfall and the percentage distribution of size classes per habitat were determined and arcsine transformed before analysis. A three-way MANOVA compared the percentages of taxa caught in diel and nocturnal traps in 1994 and 1995 in each of the 4 main enclosure habitats. A second three-way MANOVA with the same independent variables (year, habitat type and trap type) compared the distribution of size classes. The total number of invertebrates caught per habitat per month were compared by a three-way ANOVA. Total dry mass of pitfall catches in different habitats in 1994 and 1995 were compared using two-way ANOVA. Dry mass of different taxa in pitfalls from August 1995 were compared using one-way MANOVA with habitat as the independent variable. All significant MANOVA's were followed by univariate post hoc ANOVAs with modified Bonferroni correction (Simes 1986) and Tukey tests were applied to significant ANOVAs.

Stepwise multiple linear regression was used to determine the influence of microclimate on invertebrate activity/abundance as measured by pitfall trap catch. The total pitfall catch per month was regressed against the mean minimum and maximum monthly temperatures and humidity in both 1994 and 1995.

4.3.6 Statistical Analysis Of Suction Trap Data

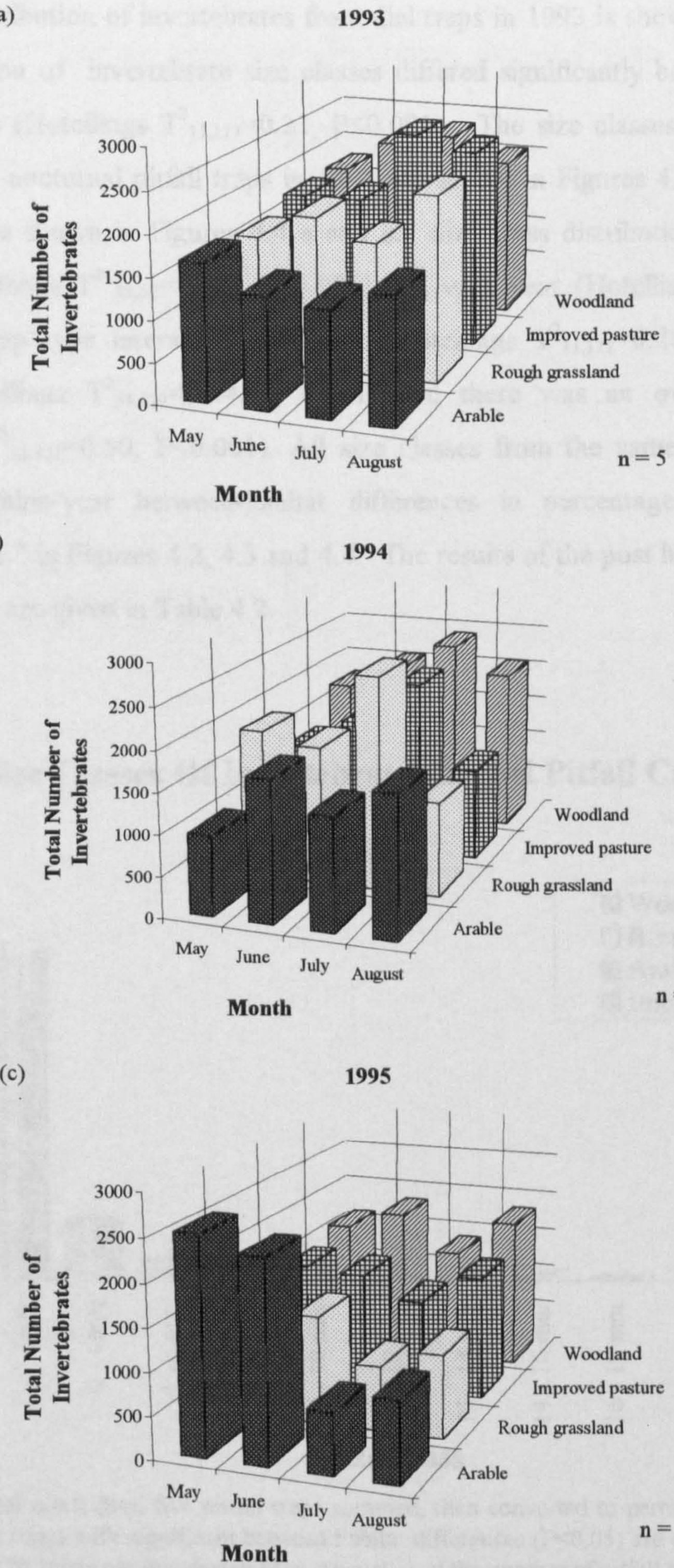
A two-way MANOVA compared the percentages of taxa suction trapped in enclosure habitats in the 1993, 1994 and 1995. A second two-way MANOVA with the same independent variables (year and habitat) compared the distribution of size classes. All significant MANOVA's were followed by univariate post hoc ANOVAs with modified Bonferroni correction (Simes 1986) where necessary and Tukey tests were applied to significant ANOVAs.

4.4 RESULTS

4.4.1 Total Number Of Invertebrates In Pitfall Traps

The total number of invertebrates per month from each of the four habitats in each of the three years 1993, 1994 and 1995 are shown in Figures 4.1a b and c respectively. Total number of invertebrates caught varied with year ($F_{2,239}$= 6.28, P<0.005) and with habitat ($F_{3,239}$=5.50, P<0.001) but not with month ($F_{2,239}$= 0.23, P>0.05). All the two-way interactions were significant: habitat and month ($F_{9,239}$=2.31, P<0.05) habitat and year ($F_{6,239}$=3.06, P<0.01), month and year ($F_{6,239}$=8.17, P<0.001). There was no overall (three-way) interaction ($F_{18,239}$=1.13, P>0.05). Woodland in 1995 had a significantly higher number of invertebrates than arable in 1993, 1994 and 1995. Arable 1995 also had a significantly higher number of invertebrates than arable in either of the previous two years. Large numbers of invertebrates were caught in arable in May and June 1995. Total numbers ranged from 2631 invertebrates from the improved pasture pitfall traps in July 1993 to 720 caught in arable in July 1995.

Figure 4.1 Total Invertebrate Catch In Diel Pitfall Traps



Total number of all invertebrates caught in pitfall traps open 24 hours per day during May-August 1993, 1994 and 1994. n = the number of pitfall traps per habitat.

4.4.2 Size Class Distribution In Pitfall Traps

Size class distribution of invertebrates from diel traps in 1993 is shown in Figure 4.2. The distribution of invertebrate size classes differed significantly between nocturnal and diel traps (Hotellings $T^2_{11,177}=0.21$, $P<0.001$). The size classes of invertebrates from diel and nocturnal pitfall traps in 1994 are shown in Figures 4.3 a and b, those from 1995 are shown in Figures 4.4 a and b. Size class distributions differed with habitat (Hotellings $T^2_{33,527}=0.49$, $P<0.001$) and with year (Hotellings $T^2_{11,177}=0.61$, $P<0.001$). Trap type interacted with year (Hotellings $T^2_{11,177}=0.28$, $P<0.001$) and habitat (Hotellings $T^2_{33,527}=0.44$, $P<0.001$) and there was an overall interaction (Hotellings $T^2_{33,527}=0.50$, $P<0.001$). All size classes from the same trap types with significant within-year between-habitat differences in percentage abundance are marked with a * in Figures 4.2, 4.3 and 4.4. The results of the post hoc univariate and TUKEY tests are given in Table 4.2.

Figure 4.2

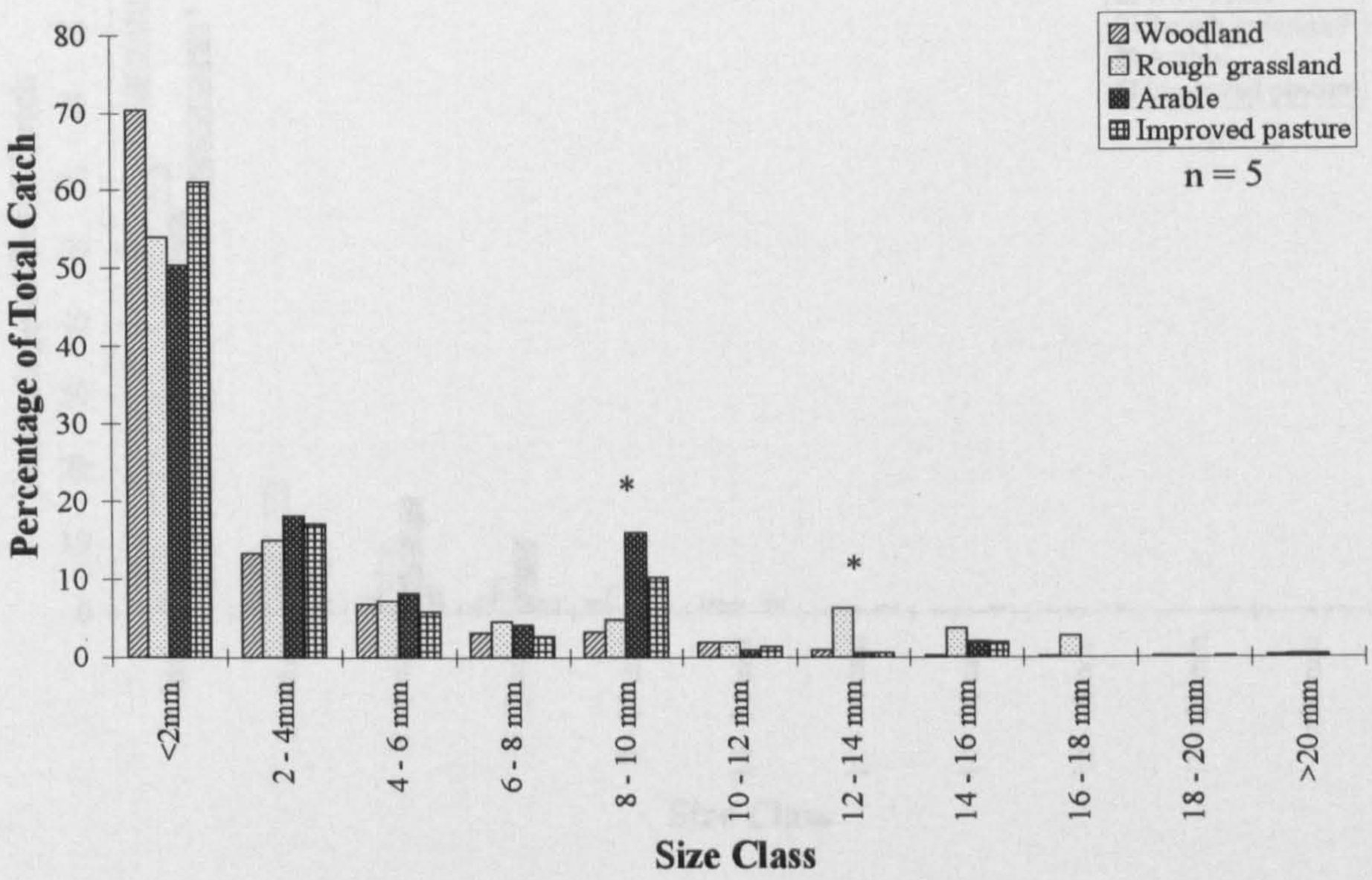
Size Classes Of Invertebrates In Diel Pitfall Catch

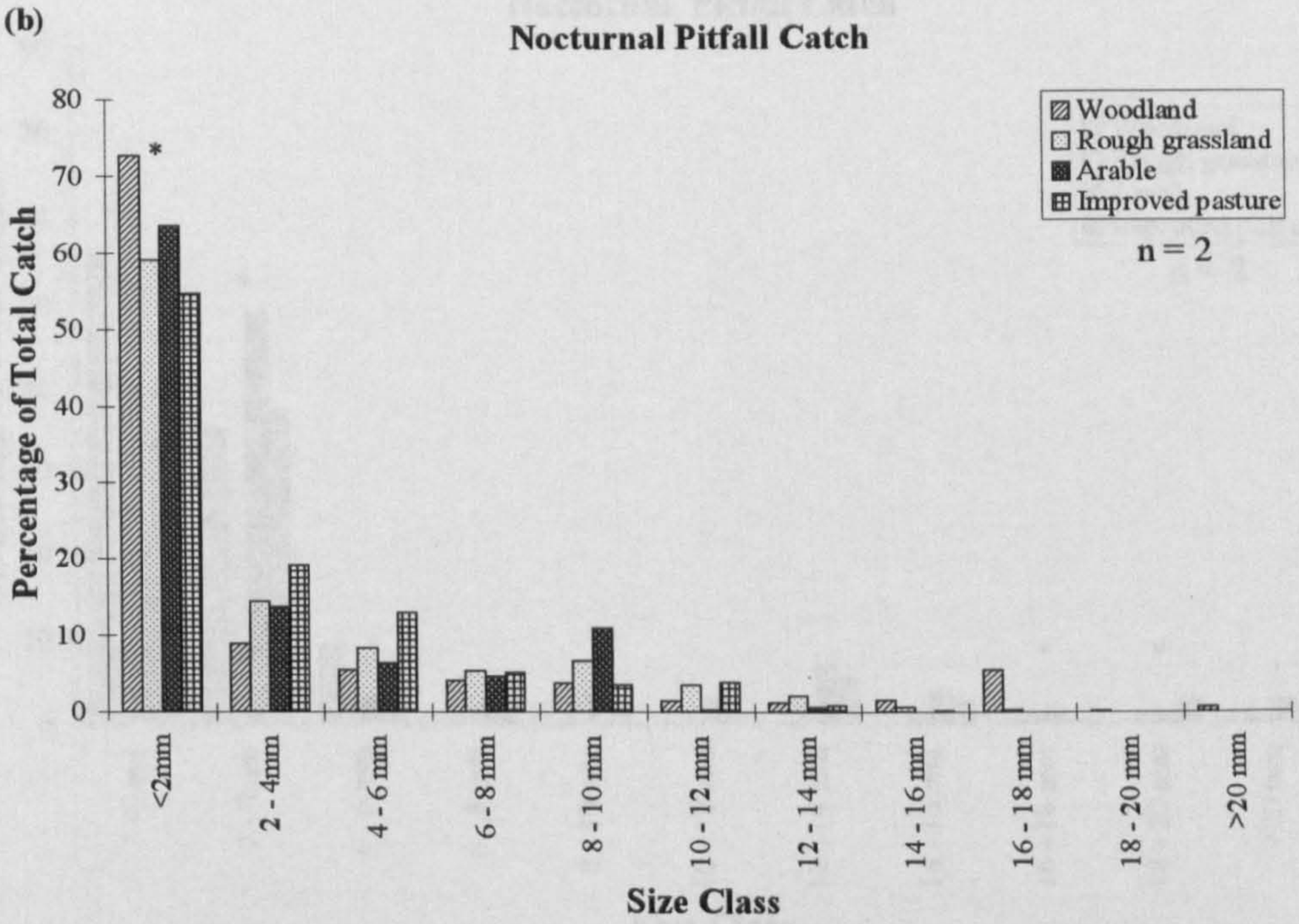
1993

Data are the total catch from five pitfall traps summed, then converted to percentages for each size category. Size classes with significant between habitat differences ($P<0.05$) are denoted with *. Diel traps were open 24 hours per day during May-August. n = the number of pitfall traps per habitat.

Figure 4.3

Size Classes Of Invertebrates In Pitfall Catch 1994

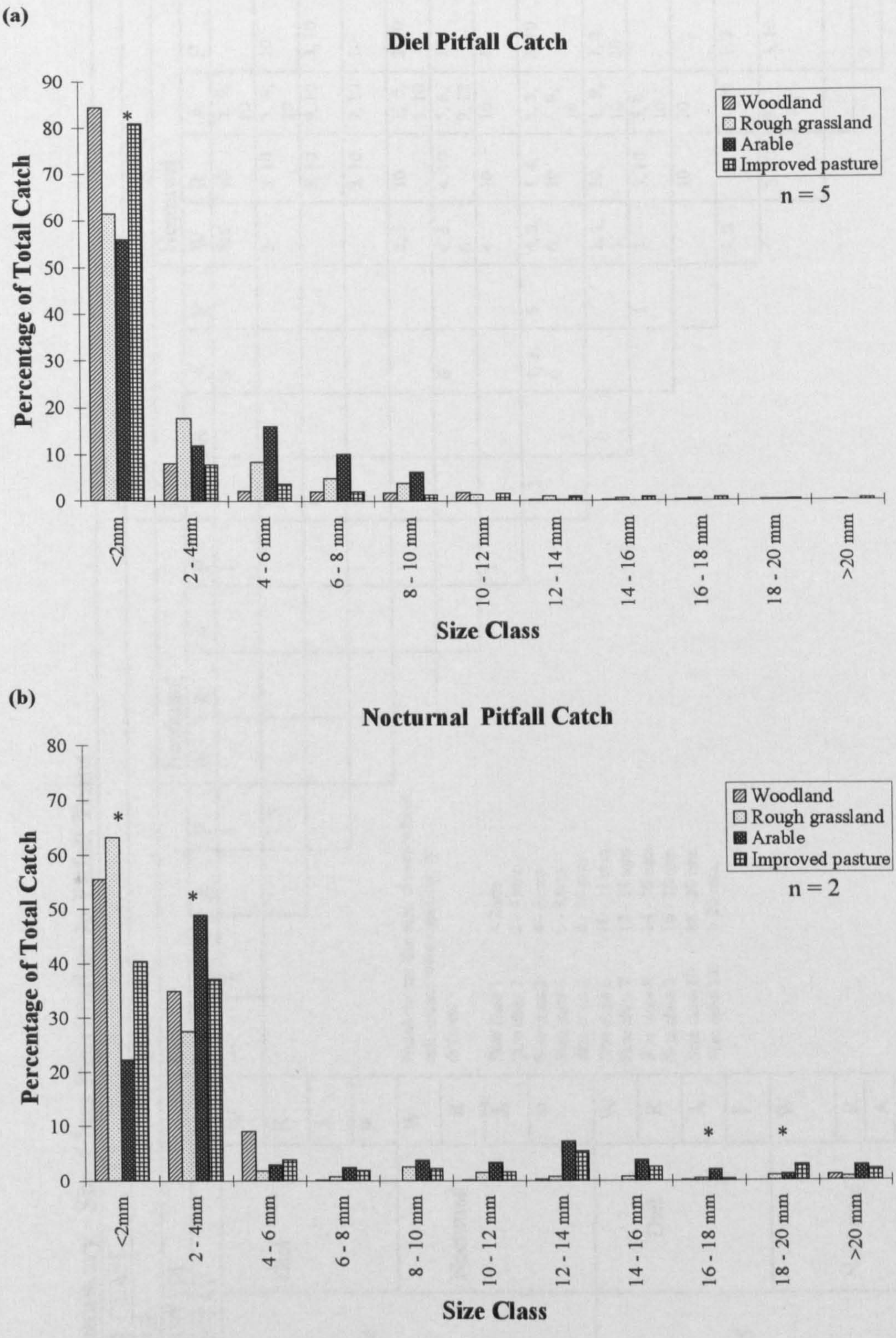




Size classes with significant between habitat differences ($P<0.05$) are denoted with *. Diel data are the total catch of five pitfall traps summed, then converted to percentages for each size category. Diel traps were open 24 hours per day during May-August. Nocturnal data are the total catch of two pitfall traps summed, then converted to percentages for each size category. Nocturnal pitfalls were open from dusk till dawn 5 days per month. n = the number of pitfall traps per habitat.

Figure 4.4

Size Classes in Pitfall Catch 1995



Diel data are the total catch of five pitfall traps summed, then converted to percentages for each size category. Size classes with significant between habitat differences ($P<0.05$) are denoted with *. Diel traps were open 24 hours per day during May-August. Nocturnal data are the total catch of two pitfall traps summed, then converted to percentages for each size category. Nocturnal pitfalls were open from dusk till dawn 5 days per month. n = the number of pitfall traps per habitat.

Table 4.2

Tukey Test Summary - Size Class Distribution In Pitfall Traps

SIZE CLASS																	
YEAR			1994								1995						
TRAPTYPE			Diel				Nocturnal				Diel				Nocturnal		
HABITAT			W	R	A	P	W	R	A	P	W	R	A	P	W	R	A	P
1994	Diel	W	—	7		5				1			6		5,6	10	5, 9, 10	
		R			7	7,5					1				5	3,10	5, 9, 10	10
		A				5					1					3,10	9,10	3,10
		P								1	1					3,10	9,10	10
	Nocturnal	W								1					2,5	10	1, 5, 9,10	2,10
		R									1		6		4,5, 6	4,10	5,6, 9,10	10
		A								1					4	10	10	10
		P									1,3		1,3, 6	6	4,5, 6	1,4, 10	3,5, 6,9, 10	3,10
1995	Diel	W										1			1,2, 3,	10	1, 9, 10	1,2, 10
		R												1	5	3,10	5,9, 10	
		A														10	10	
		P													1,2		1,10	1,2
	Nocturnal	W														3,10	9, 10,	3,10
		R																
		A																9
		p																

Numbers are the size classes whose cell means were significantly different

Size class 1	< 2mm
Size class 2	2 - 4 mm
Size class 3	4 - 6 mm
Size class 4	6 - 8 mm
Size class 5	8 - 10 mm
Size class 6	10 - 12 mm
Size class 7	12 - 14 mm
Size class 8	14 - 16 mm
Size class 9	16 - 18 mm
Size class 10	18 - 20 mm
Size class 11	> 20 mm

W = woodland, P = pasture, Rough grassland, A = arable. Diel = Pitfall traps open 24 hours all May-September. Nocturnal = Pitfall traps open from dawn to dusk for 5 days per month.

Size class categories were log normally distributed, with up to 80% of invertebrates caught in the smallest size category (size class 1). In general, woodland had a higher percentage of small invertebrates (size classes 1 - 3) than all other habitats and arable had a higher percentage of the middle size categories (classes 5, 6 and 7) than all other habitats. Nocturnal pitfall traps in 1995 had a higher percentage of the large invertebrates (16-20mm) than the diel pitfall catch.

In the 1994 diel pitfall traps in arable had significantly more size class 5 invertebrates than any other habitat ($F_{3,187}$=2.17, P<0.005). Rough grassland diel traps in 1994 significantly more size class 7 invertebrates than any other habitat ($F_{3,187}$=5.29, P<0.01). In 1994 nocturnal pitfall traps in arable and woodland had significantly higher percentages of size class 1 invertebrates than the rough grassland and improved pasture ($F_{3,187}$=3.26, P<0.05). In 1995 nocturnal pitfall traps in woodland had significantly more size class 3 invertebrates than all other habitats ($F_{3,187}$=3.16, P<0.05). In 1995 nocturnal traps in arable had significantly more size class 2 and 9 invertebrates than traps in all other habitats ($F_{3,187}$=3.16, P=0.05; $F_{3,187}$=6.35, P<0.001 respectively). Nocturnal pitfall catch in 1995 had significantly more size class 2 invertebrates and significantly less small (size class 1) invertebrates than the diel pitfall catch ($F_{3,187}$=10.58, P<0.001).

4.4.3. Percentage Abundance Of Taxa In Pitfall Traps

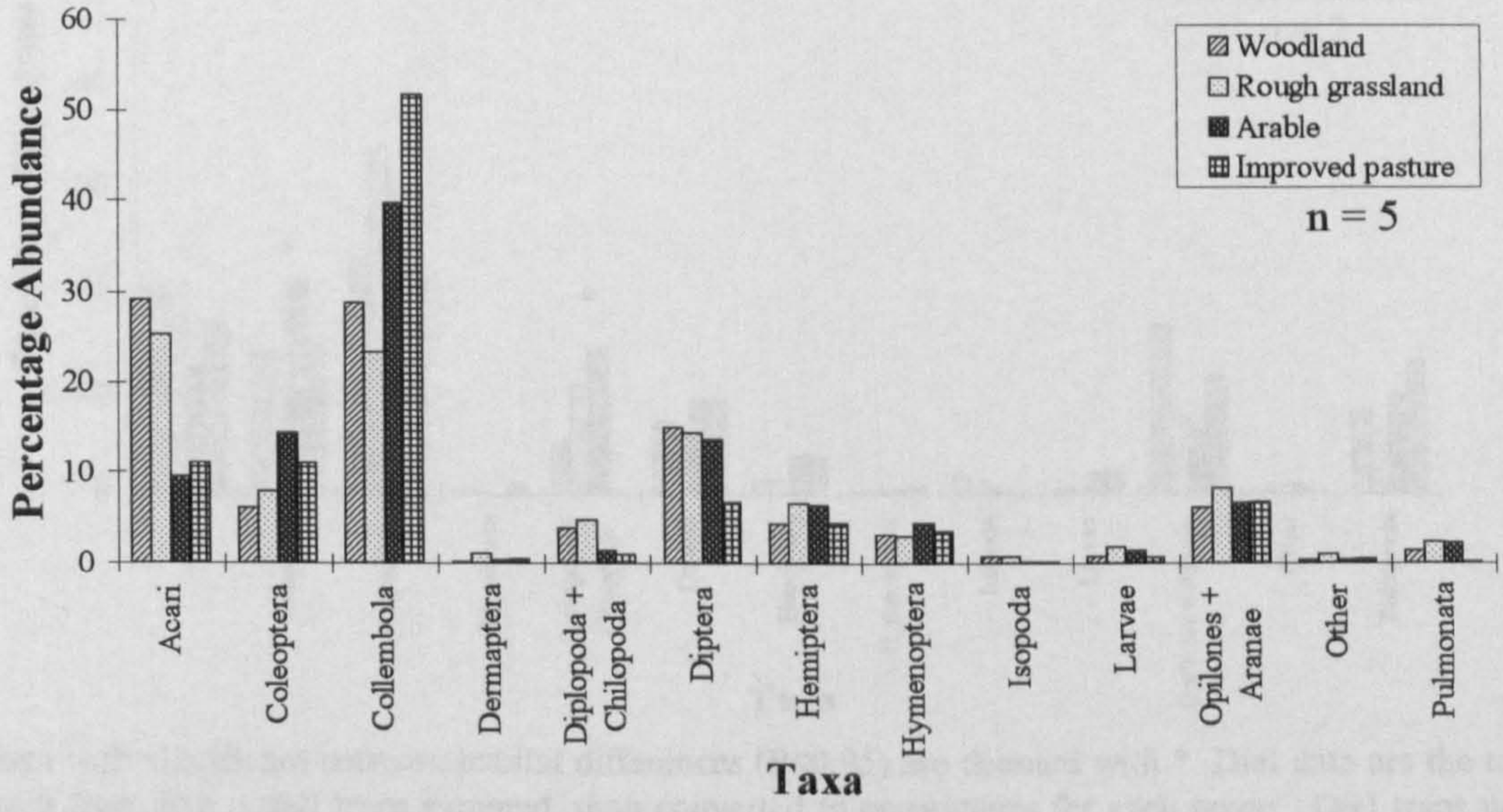
Percentage abundance of invertebrates from diel traps in 1993 are shown in Figure 4.5. Percentage abundance of invertebrate taxa differed between nocturnal and diel traps (Hotellings $T^2_{13,175}$=0.38, P<0.001). Percentage abundance of invertebrate taxa from diel and nocturnal pitfalls in 1994 are shown in Figures 4.6 a and b, those from 1995 are shown in Figures 4.7 a and b. Percentage abundance of invertebrate taxa differed with habitat (Hotellings $T^2_{39,521}$=1.16, P<0.001) and with year (Hotellings $T^2_{13,175}$=0.64, P<0.001). Trap type also interacted with year (Hotellings $T^2_{13,175}$=0.52, P<0.001) and habitat (Hotellings $T^2_{39,521}$=0.33, P<0.05) and there was a significant overall interaction (Hotellings $T^2_{39,521}$=0.39, P<0.005).

All taxa from the same trap types with significant within-year, between-habitat differences in percentage abundance are given an asterisk (*) in Figures 4.6 and 4.7. The results of the post hoc univariates and TUKEYS are given in Table 4.3. Percentage abundance of Coleoptera in arable traps was higher than in other habitats ($F_{3,187}$=5.71, P<0.001) during nocturnal and diel trapping in 1994. In the diel and nocturnal pitfall catch in 1995 woodland had a higher percentage of Acari than all other habitats ($F_{3,187}$=25.39, P<0.0001) and improved pasture had a higher percentage Diptera than any other habitat ($F_{3,187}$=3.69, P<0.01). In 1995 diel pitfall traps in arable had a higher percentage of Hemiptera and a lower percentage Collembola than all other habitats ($F_{3,187}$=0.29 P<0.05; $F_{3,187}$=30.69 P<0.0001). In nocturnal pitfall traps in 1994 improved pasture had a significantly lower percentage abundance of Diplopoda and Chilopoda than all other habitats($F_{3,187}$=8.57, P<0.0001). In nocturnal traps 1995 rough grassland and woodland had significantly more Isopoda than arable and improved pasture ($P_{3,187}$=9.65, P<0.001).

Figure 4.5

Percentage Abundance Of Taxa In Diel Pitfall Traps

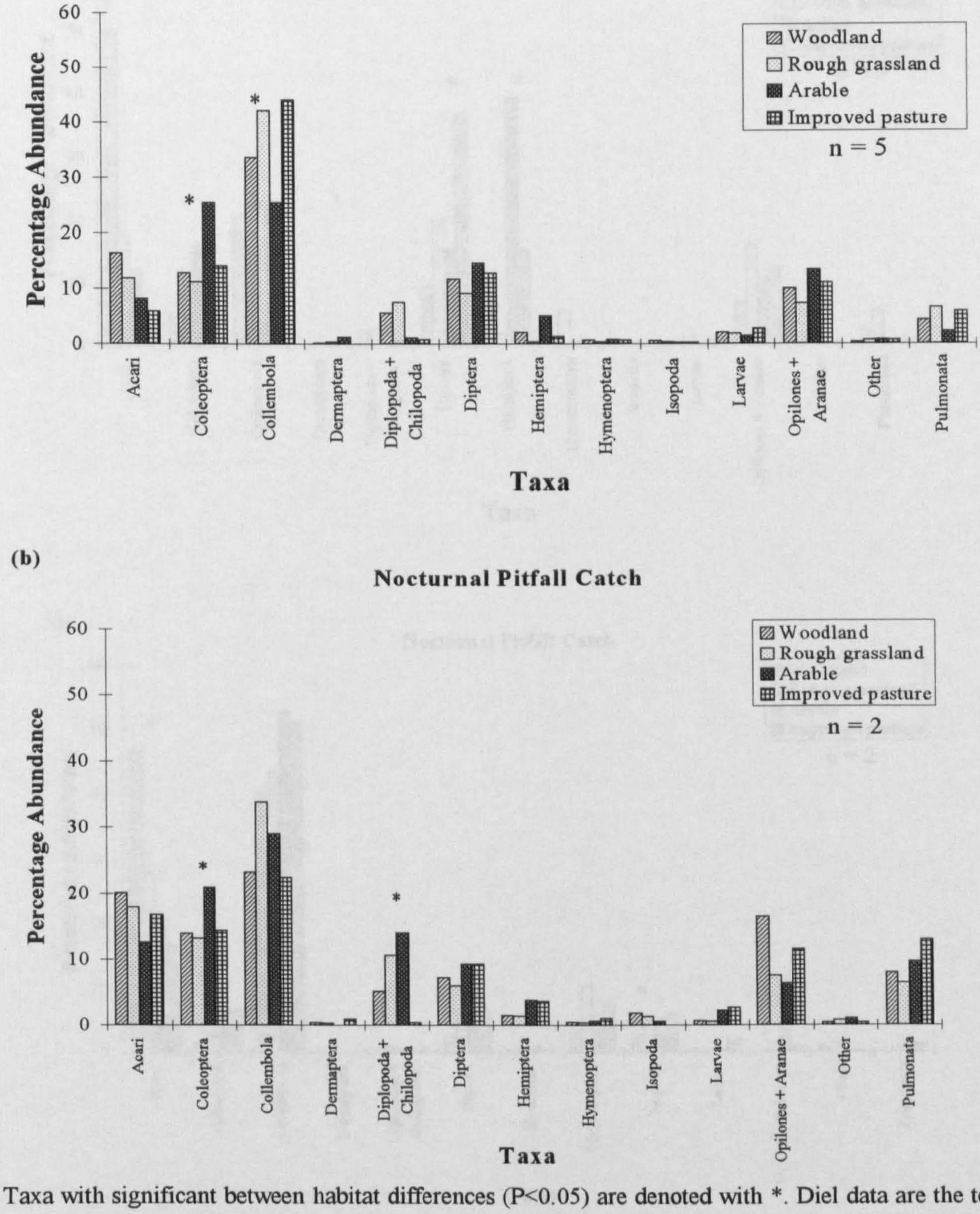
1993



Data are the total catch of five pitfall traps summed, than converted to percentages for each taxon. Taxa with significant between habitat differences (P<0.05) are denoted with *. Diel traps were open 24 hours per day during May-August. Other = all other invertebrate taxa caught in pitfall traps. Insect Larvae = mostly consisted of Coleopteran, Dipteran and Lepidopteran larvae. n = number of pitfalls per habitat.

Figure 4.6

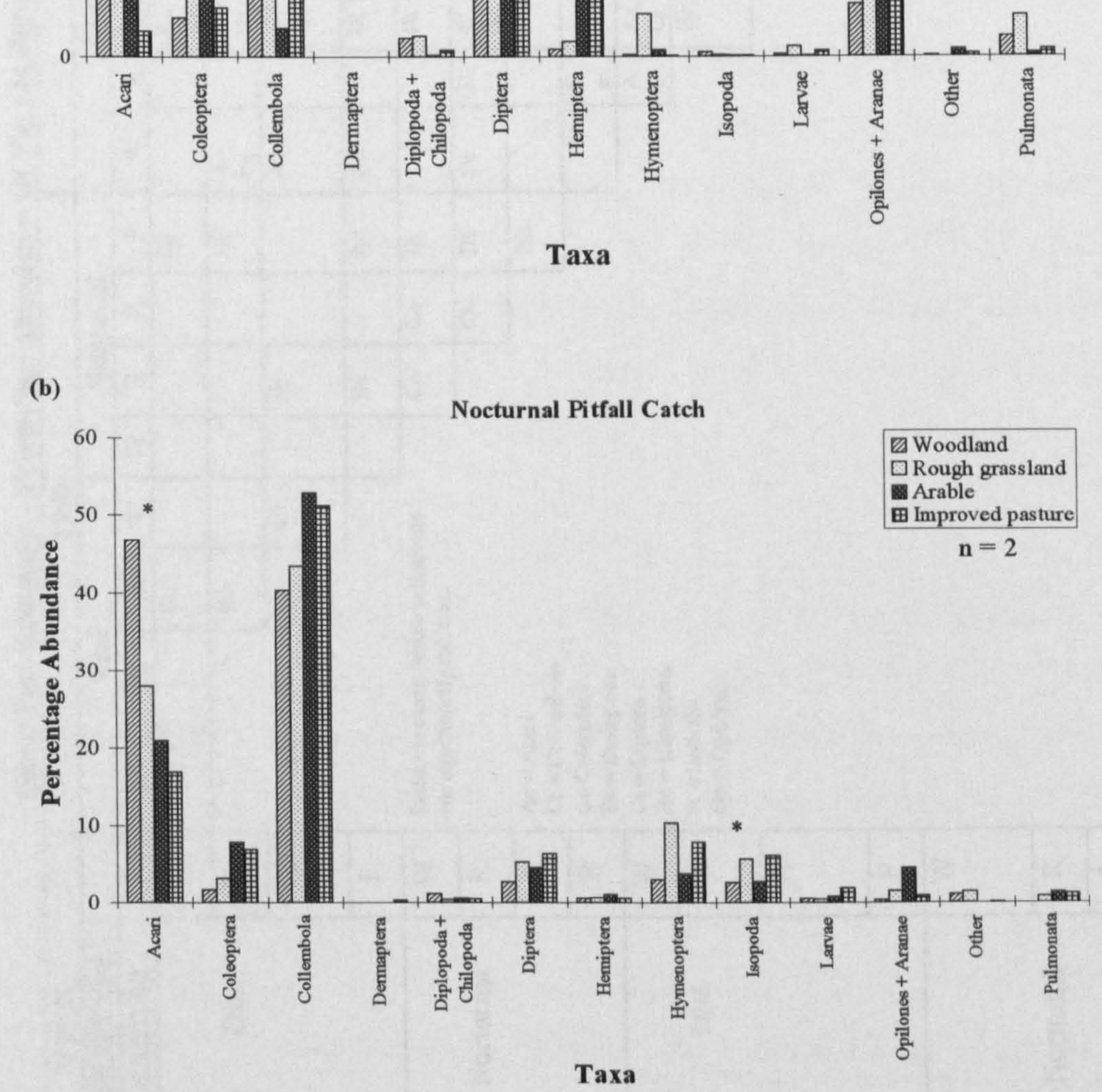
Percentage Abundance of Taxa in Pitfall Catch 1994



Taxa with significant between habitat differences ($P<0.05$) are denoted with *. Diel data are the total catch from five pitfall traps summed, than converted to percentages for each taxon. Diel traps were open 24 hours per day during May-August. Nocturnal data are the total catch of two pitfall traps summed, then converted to percentages for each taxon. Nocturnal pitfalls were open from dusk till dawn 5 days per month. n = the number of pitfall traps per habitat. Other = all other invertebrate taxa caught in pitfall traps. Insect Larvae = mostly consisted of Coleopteran, Dipteran and Lepidopteran larvae.

Figure 4.7

Percentage Abundance Of Taxa In Pitfall Catch -1995



Taxa with significant between habitat differences ($P<0.05$) are denoted with *. Diel data are the total catch from five pitfall traps summed, than converted to percentages for each taxon. Diel traps were open 24 hours per day during May-August. Nocturnal data are the total catch of two pitfall traps summed, then converted to percentages for each taxon. Nocturnal pitfalls were open from dusk till dawn 5 days per month. n = the number of pitfall traps per habitat. Other = all other invertebrate taxa caught in pitfall traps. Insect Larvae = mostly consisted of Coleopteran, Dipteran and Lepidopteran larvae.

Table 4.3

Tukey Test Summary - Percentage Abundance Of Taxa In Pitfall Traps

YEAR			1994								1995							
TRAPTYPE			Diel				Nocturnal				Diel				Nocturnal			
HABITAT			W	R	A	P	W	R	A	P	W	R	A	P	W	R	A	P
1994	Diel	W			Co					Di			Ac, Di, He	Ac			Di	Di
		R			Co					Di	Ac, Co		Co, Cl, Di, He	Di	Ac, Co	Ac, Co	Di	Di
		A				Co		Di			Ac		He		Ac, Co, Is, Op	Ac		
		P						Di		Ac	Ac		Cl, He		Ac, Co	Ac	Is	Is
	Nocturnal	W						Co	Co	Di			Di, He		Op	Op	Di	Di, Op
		R							Co	Di	Di	Di	Cl, Di, He	Di	Di	Di	Di	Di
		A								Di			Di, He		Di	Ac, Di	Di	Di
		P										H e		Ac, Co	Op		Cl	
1995	Diel	W										A c	Ac, He, Cl	Ac, Dt			Ac	Cl
		R											He, Cl	Dt	Ac, Co, Op	Ac, Op		Cl, Op
		A												He Cl Dt	Ac, He, Is, Op	Ac, Cl, He, Op	Cl, He	Cl, He, Op
		P													Ac,Dt Is, Op	Ac, Dt, Op		Cl
	Nocturnal	W														Ac	Ac, Co, Is, Op	Ac, Is
		R															Co, Is	
		A																Is
		P																

Letters are taxa whose cell means are significantly different

Ac = Acari
Cl = Collembola
Co Coleoptera
Di = Diplopoda
Dt = Diptera
He = Hemiptera
Is = Isopoda
Op = Opilones

W = woodland, P = pasture, Rough grassland, A = arable. Diel = Pitfall traps open 24 hours all May-September. Nocturnal = Pitfall traps open from dawn to dusk for 5 days per month

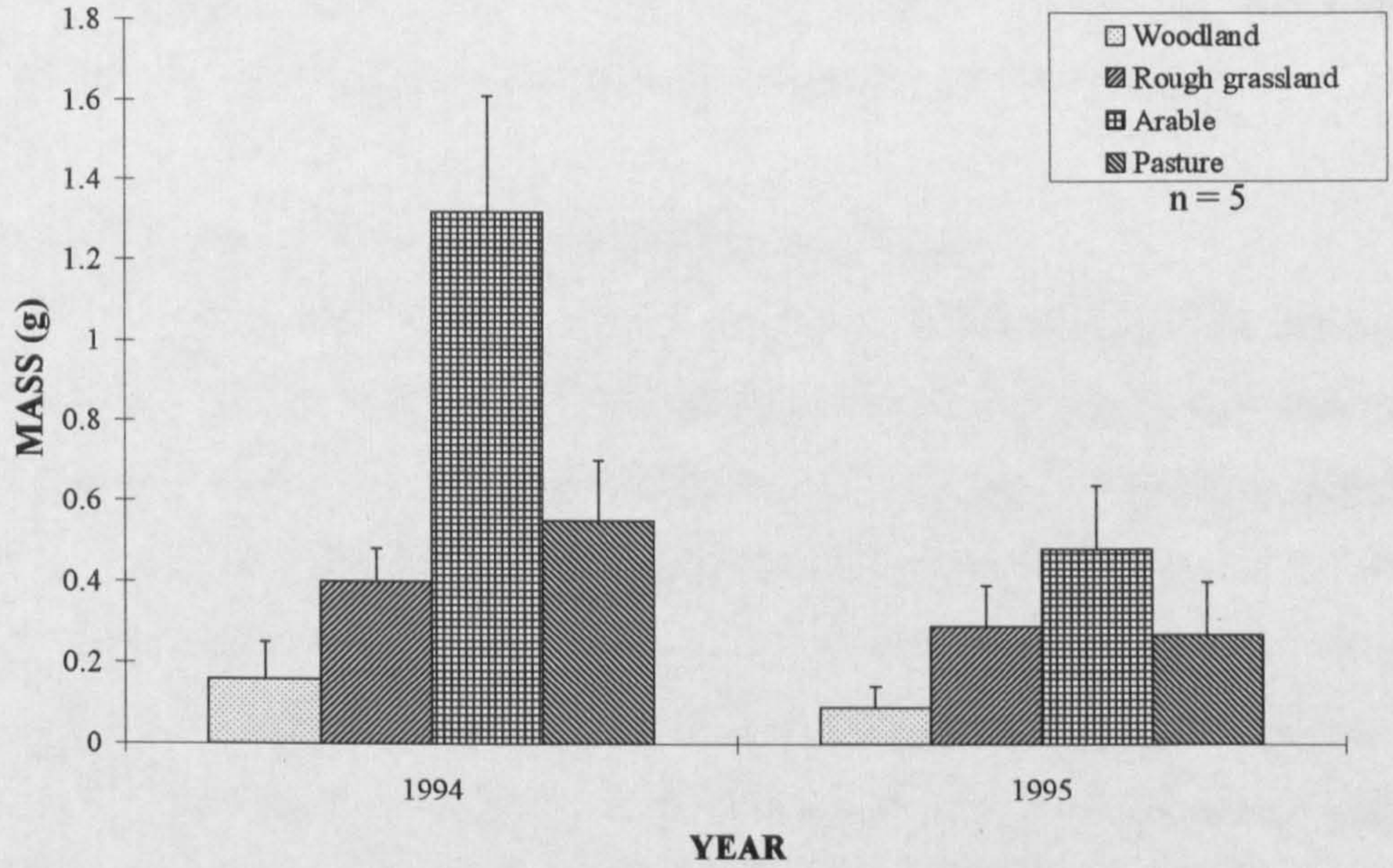
The patterns for percentage abundance of taxa in diel and nocturnal traps in 1995 differed markedly. Diel traps in 1995 had high percentage abundances of Hemiptera in arable ($F_{1,187}$=7.41, P<0.01), of Acari in woodland ($F_{1,187}$= 16.84, P<0.0001) and of Diptera in improved pasture ($F_{1,187}$=11.56, P<0.001). The nocturnal pitfall catches of 1995 had higher percentage abundance of Collembolla ($F_{1,187}$=13.51, P<0.001) and of Isopoda ($F_{1,187}$=4.94, P<0.05) than the diel pitfall catches. There were higher percentages of Opilones and Aranae in diel pitfall traps than in nocturnal traps in 1995 ($F_{1,187}$=13.02, P<0.0001).

In 1995, both diel and nocturnal pitfalls had lower percentage abundance of Coleopterans than in 1994 ($F_{1,187}$=11.29, P<0.001). From 1994 to 1995 there were large percentage abundance increases in Hemiptera and Diptera mainly in arable and improved pasture habitats in main pitfall traps ($F_{1,187}$=10.09, P<0.002; $F_{1,187}$=11.56, P<0.001). From 1994 to 1995 the percentage abundance of Isopoda Collembola and Acari in nocturnal traps increased ($F_{1,187}$=9.65, P<0.0001; $F_{1,187}$=30.69, P<0.0001; $F_{1,187}$=8.67, P<0.0001). From 1994 to 1995 the percentage abundance of Opilones + Aranae and Chilopoda + Diplopoda in nocturnal pitfall traps decreased ($F_{1,187}$=3.54, P<0.016; $F_{1,187}$=38.75, P<0.0001)

4.4.4 Dry Mass Of Taxa In Pitfall Traps

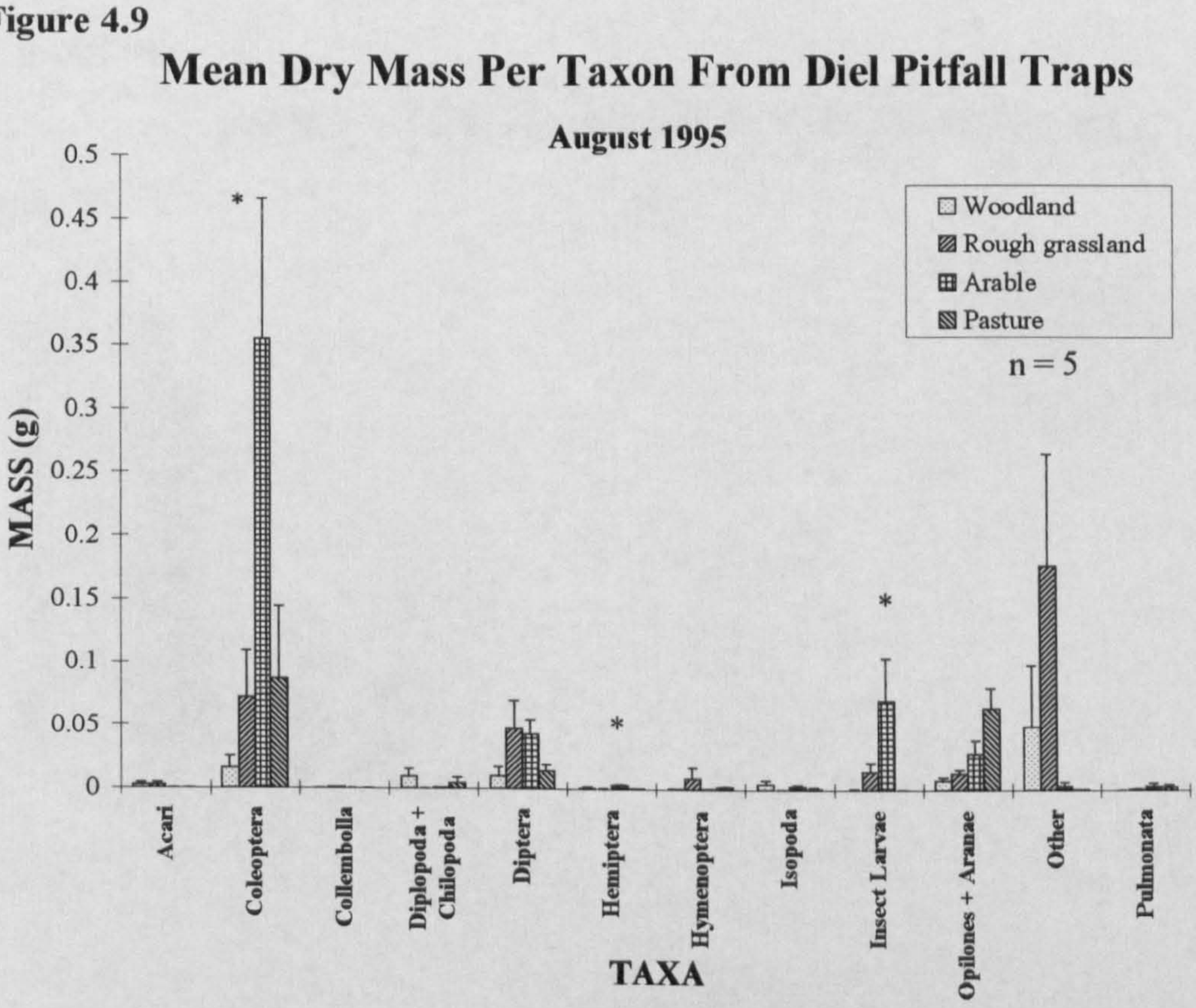
The mean dry mass per pitfall per month in both years are shown in Figure 4.8. Pitfall dry mass in 1994 was nearly twice that of 1994 ($F_{1,39}$=9.789, P<0.005). Arable had significantly higher mass per pitfall than all other habitats ($F_{3,39}$=7.276, P<0.001). There was a significant interaction between the two factors (year and habitat) because mass of trapped invertebrates from the arable habitat in 1994 were significantly greater than those from all other habitats in both years ($F_{3,39}$=5.363, F<0.005). Dry mass of invertebrates from arable in 1994 was more than double that of all other habitats in 1994 and all habitats, including arable, in 1995. The mean dry mass per pitfall of each taxon from diel pitfall traps in August 1995 are shown in Figure 4.9.

Figure 4.8



Data are mean mass of invertebrates per pitfall per month per habitat, +/- one standard error of the mean. n = the number of pitfall traps per habitat.

Figure 4.9



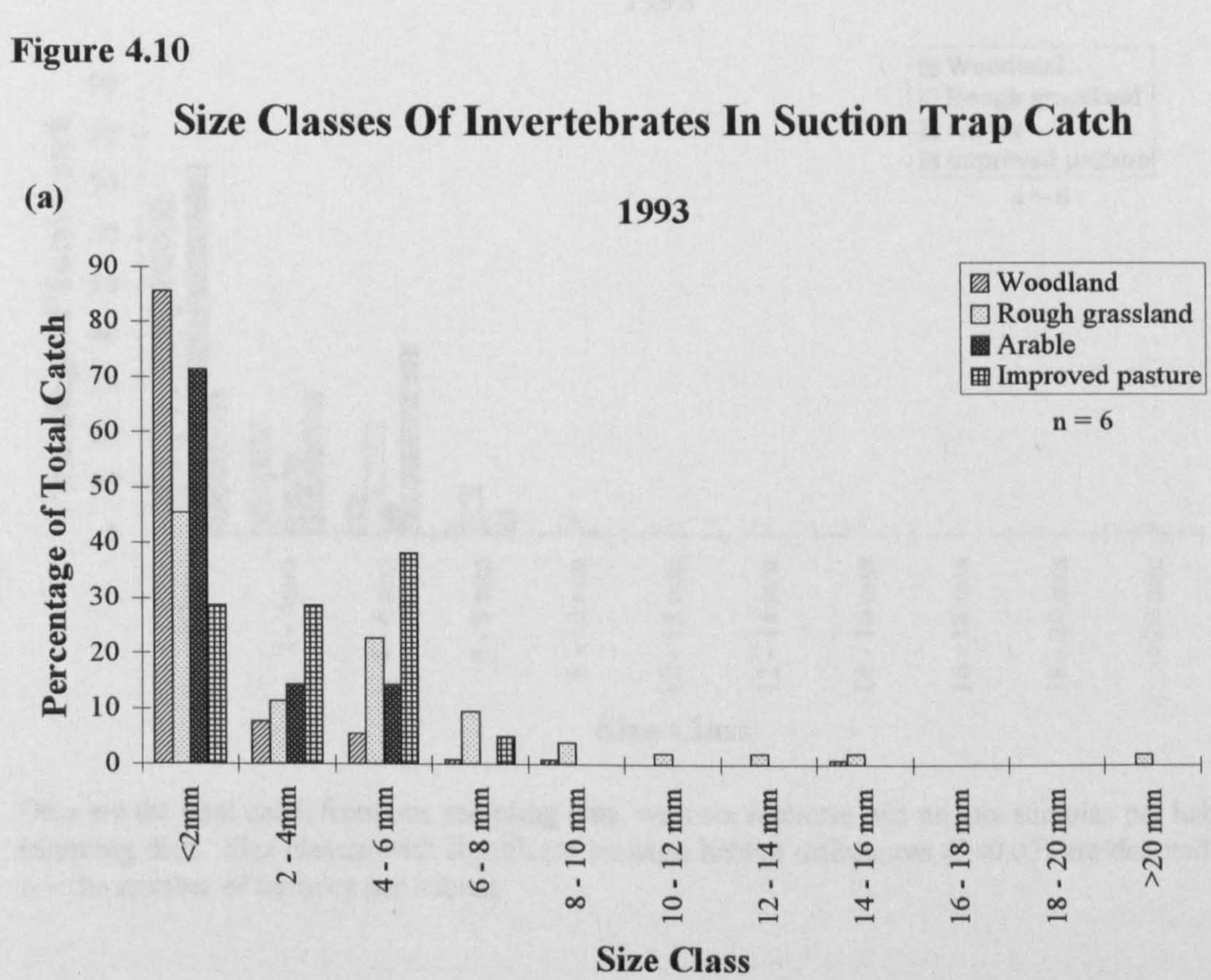
Data are mean of five pitfall traps per habitat for the month of August +/- one standard error of the mean. Other = all other invertebrate taxa caught in pitfall traps. Insect Larvae = mostly consisted of Coleopteran, Dipteran and Lepidopteran larvae. n = the number of pitfall traps per habitat.

Taxon dry mass differed between habitats (Hotellings $T^2_{36,11}=65.22$, $P<0.001$). Arable had a significantly greater mass of Coleoptera and insect larvae than all other habitats ($F_{3,16}=5.36$, $P<0.01$; $F_{3,16}=3.94$, $P<0.05$ respectively). Arable also had a significantly higher mass of Hemiptera than rough grassland ($F_{3,16}=4.56$, $P<0.05$).

4.4.5. Size Class Of Suction Trapped Invertebrates

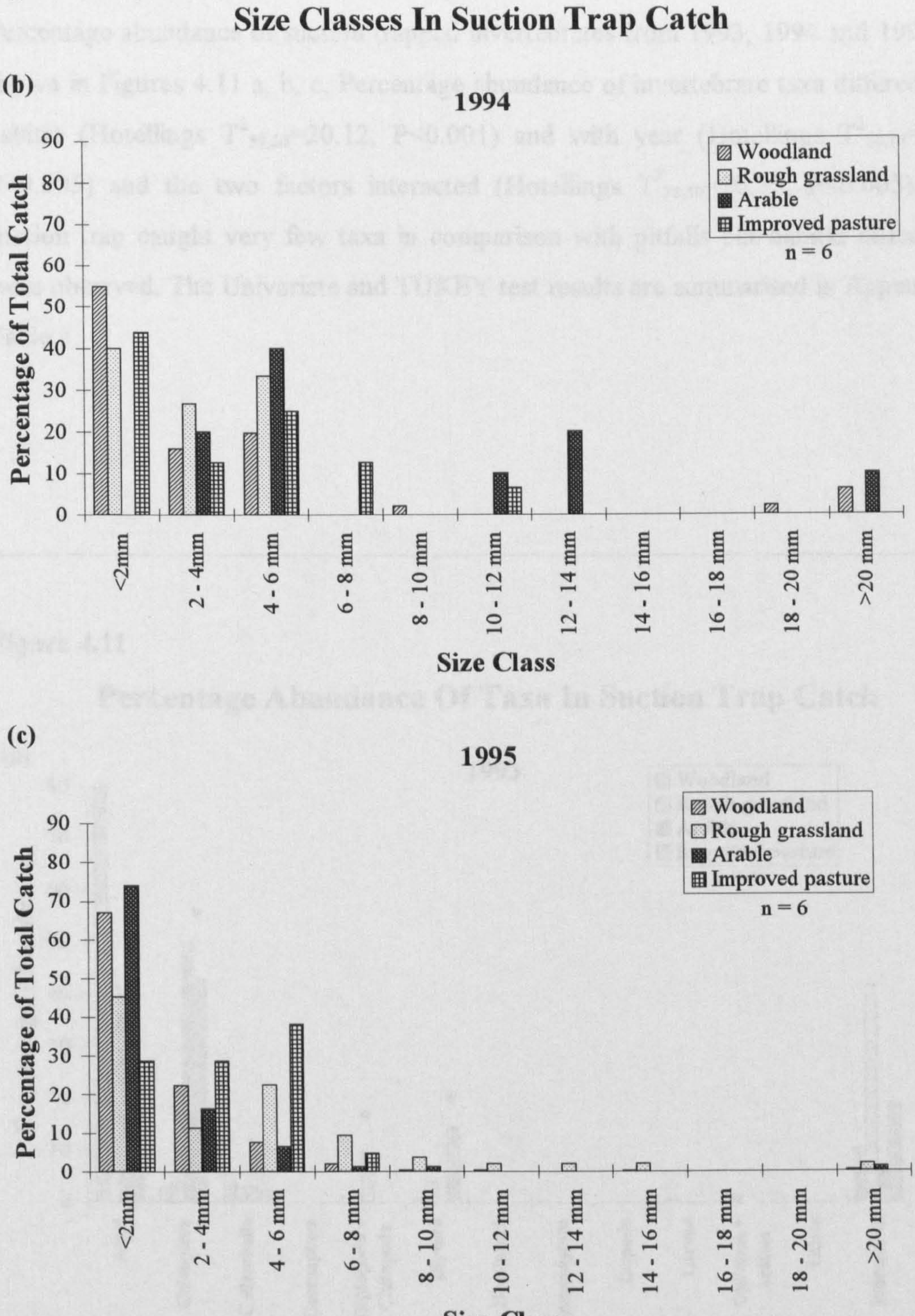
Total numbers of invertebrates above 0.3mm in length caught with the suction trap were 259 in 1993, 93 in 1994 and 889 in 1995. Size class distributions of suction trapped invertebrates are shown in Figures 4.10 a, b, and c. Size class distributions of suction trapped taxa did not differ between years (Hotellings $T^2_{20,24}=1.72$, $P>0.05$) or between habitats (Hotellings $T^2_{30,35}=1.72$, $P>0.05$) and there was no significant interaction (Hotellings $T^2_{60,68}=4.44$, $P>0.05$). Size class categories tended towards log normal distributions in all three years with up to 85% of invertebrates caught in the smallest two size classes. Less than 5% of invertebrates caught in 1993 and 1995 were larger than 8 mm. In 1994 arable had no invertebrates in size class 1 (<2mm).

Figure 4.10



Data are the total catch from three sampling dates, with six replicate two minute samples per habitat per sampling date. Size classes with significant between habitat differences ($P<0.05$) are denoted with *. n = the number of samples per habitat per sampling date.

Figure 4.10 (Cont..)



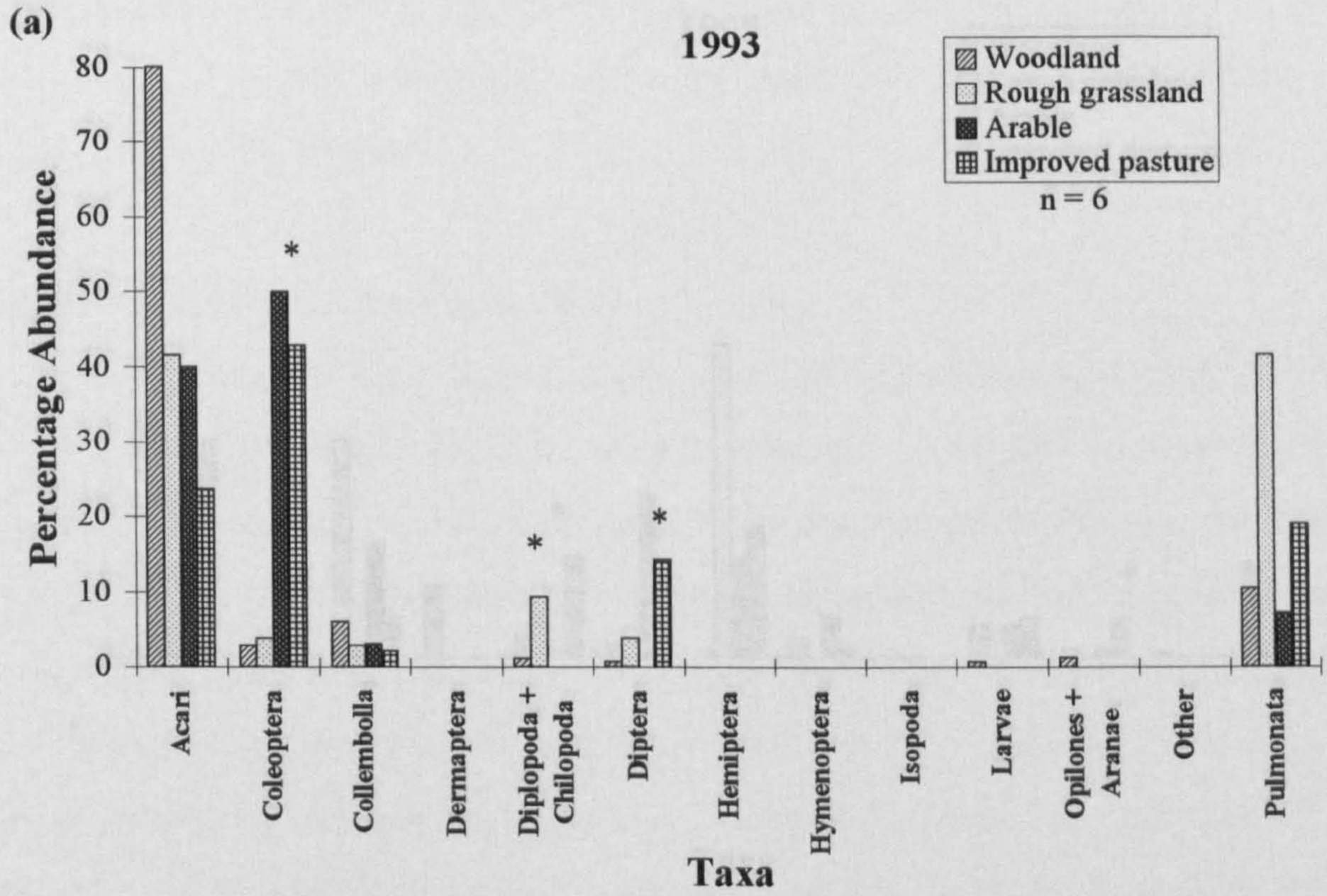
Data are the total catch from one sampling date, with six replicate two minute samples per habitat per sampling date. Size classes with significant between habitat differences ($P<0.05$) are denoted with *.
n = the number of samples per habitat

4.4.6 Percentage Abundance Of Taxa In Suction Traps

Percentage abundance of suction trapped invertebrates from 1993, 1994 and 1995 are shown in Figures 4.11 a, b, c. Percentage abundance of invertebrate taxa differed with habitat (Hotellings $T^2_{39,26}=20.12$, $P<0.001$) and with year (Hotellings $T^2_{26,18}=9.79$, $P<0.005$) and the two factors interacted (Hotellings $T^2_{78,50}=20.55$, $P<0.005$). The suction trap caught very few taxa in comparison with pitfalls but habitat differences were observed. The Univariate and TUKEY test results are summarised in Appendix B Table 1.

Figure 4.11

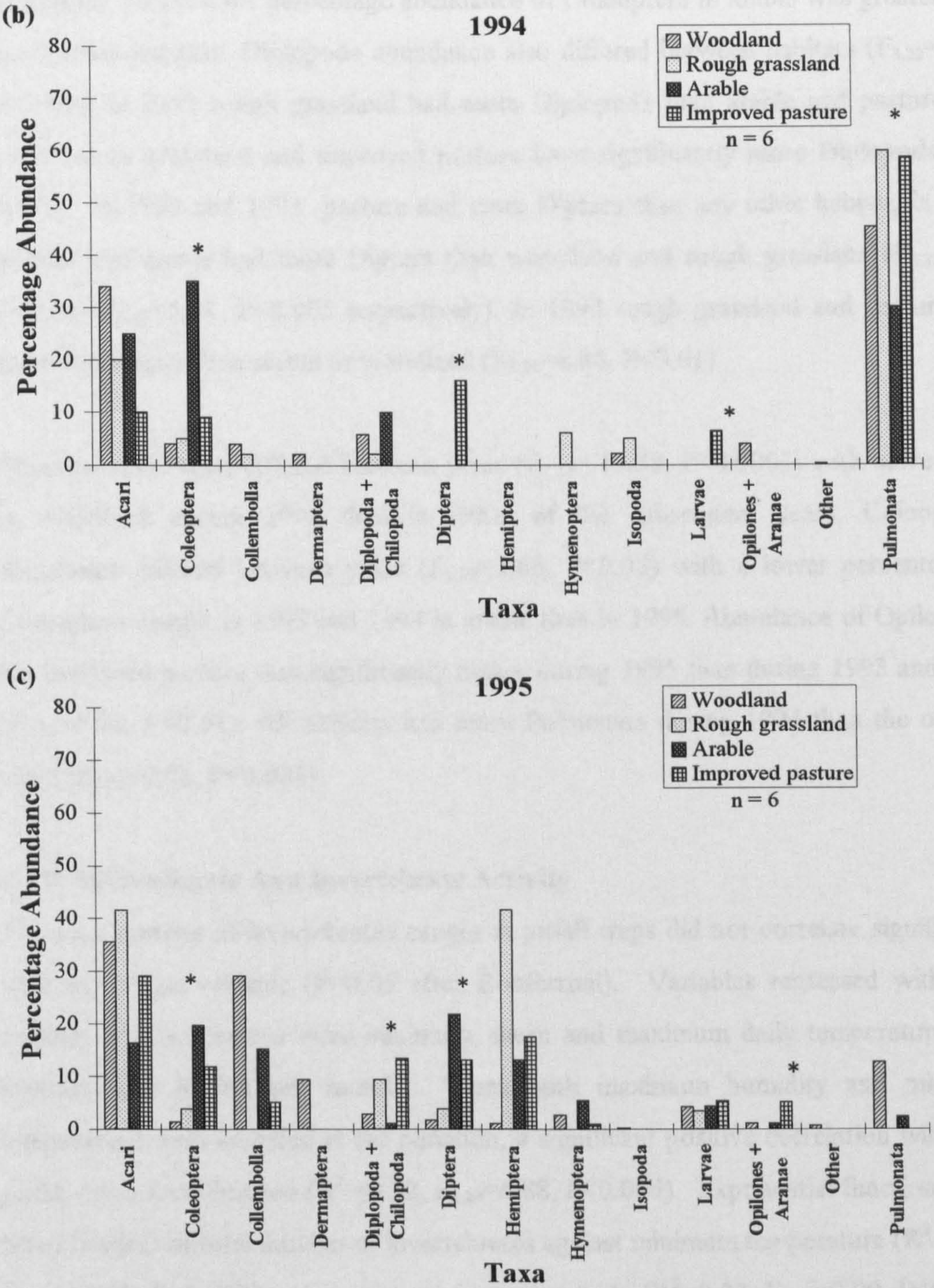
Percentage Abundance Of Taxa In Suction Trap Catch



Data are the total catch from three dates of sampling, with six replicate two minute samples per habitat per sampling date. Taxa with significant between habitat differences ($P<0.05$) are denoted with *. Other = all other invertebrate taxa caught in pitfall traps. Insect Larvae = mostly consisted of Coleopteran, Dipteran and Lepidopteran larvae. n = the number of samples per habitat per sampling date.

Figure 4.11

Percentage Abundance Of Taxa In Suction Trap Catch



Data are the total catch from one sampling date, with six replicate two minute samples per habitat per sampling date. Taxa with significant between habitat differences ($P<0.05$) are denoted with *. Other = all other invertebrate taxa caught in pitfall traps. Insect Larvae = mostly consisted of Coleopteran, Dipteran and Lepidopteran larvae. n = the number of samples per habitat

Coleoptera differed between habitats ($F_{3,22}$=3.47, P<0.05) in 1993 and in 1995; arable and pasture had significantly higher numbers of Coleoptera than woodland and rough grassland. In 1994 the percentage abundance of Coleoptera in arable was greater than in all other habitats. Diplopoda abundance also differed between habitats ($F_{3,22}$=3.39, P<0.05); in 1993 rough grassland had more Diplopoda than arable and pasture. In 1995 rough grassland and improved pasture have significantly more Diplopoda than arable. In 1993 and 1994 pasture had more Diptera than any other habitat, in 1995 pasture and arable had more Diptera than woodland and rough grassland ($F_{3,22}$=8.3, P<0.001;$F_{3,22}$=5.91, P<0.005 respectively). In 1994 rough grassland and pasture had more Pulmonata than arable or woodland ($F_{3,22}$=4.86, P<0.01).

Abundance of Acari differed between years ($F_{2,22}$= 13.38, P<0.0001) with more Acari in woodland during 1993 than in either of the subsequent years. Coleopteran abundance differed between years ($F_{2,22}$=3.66, P<0.05) with a lower percentage of Coleoptera caught in 1993 and 1994 in arable than in 1995. Abundance of Opilones in the improved pasture was significantly higher during 1995 than during 1993 and 1994 ($F_{2,22}$=6.21, P<0.01). All habitats had more Pulmonata during 1994 than the other 2 years ($F_{2,22}$=9.02, P<0.001).

4.4.7. Microclimate And Invertebrate Activity

The total number of invertebrates caught in pitfall traps did not correlate significantly with any single variable (P>0.05 after Bonferroni). Variables regressed with total number of invertebrates were minimum, mean and maximum daily temperatures and humidity per habitat per month. When both maximum humidity and minimum temperature were included in the equation, a significant positive correlation with total pitfall catch was obtained (R^2 =0.32, $F_{2,29}$=6.88, P<0.005). Exponential functions were fitted to plots of total number of invertebrates against minimum temperature (R^2 =0.28, $F_{1,30}$=11.38, P<0.005) and against maximum humidity (R^2=0.23, $F_{1,30}$=9.09, P<0.005). Total number of invertebrates caught relative to the minimum temperature and maximum humidity are shown in Figures 4.12 and 4.13 respectively. The exponential and linear regression equations and R^2 values are shown on each graph.

Figure 4.12

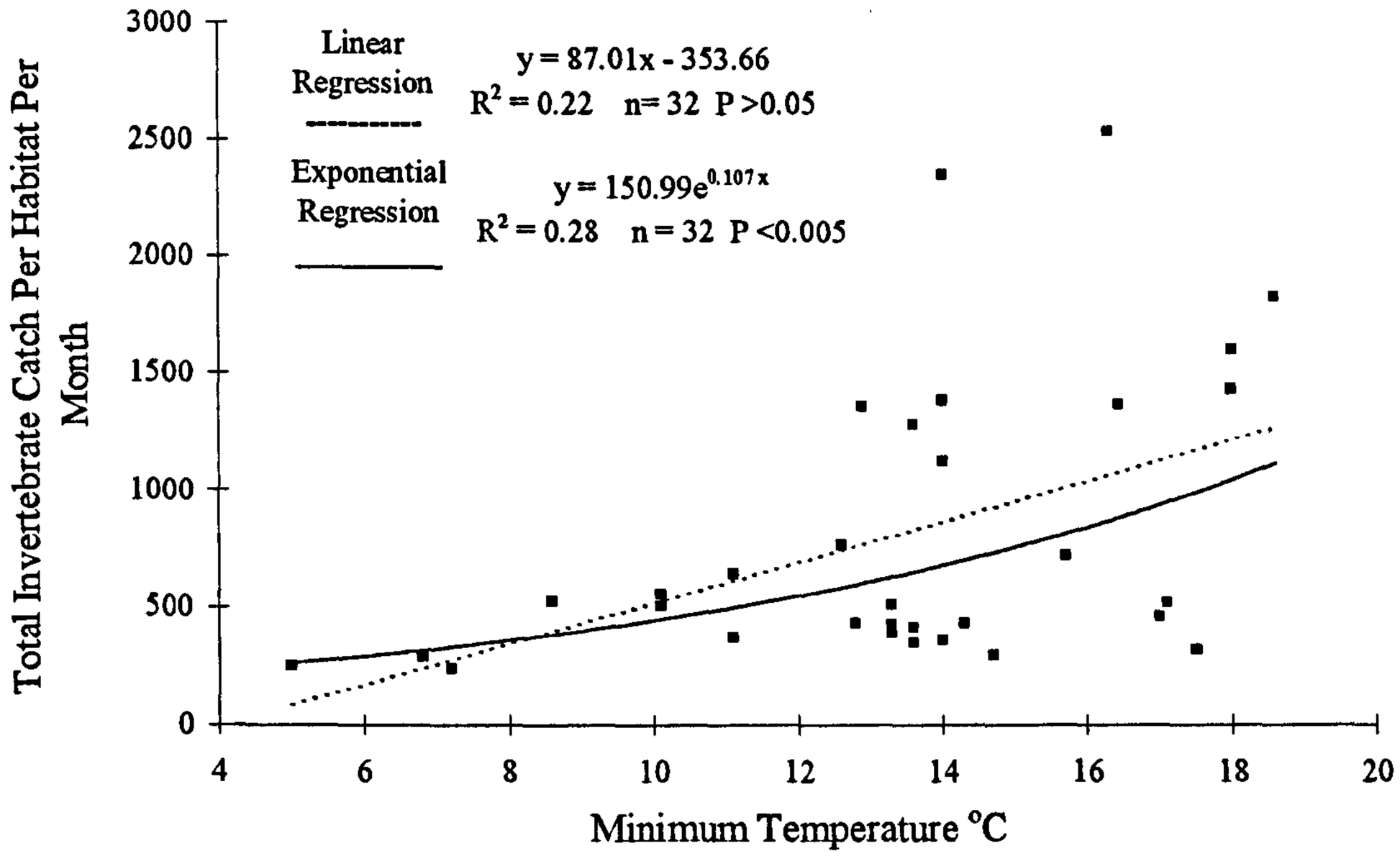
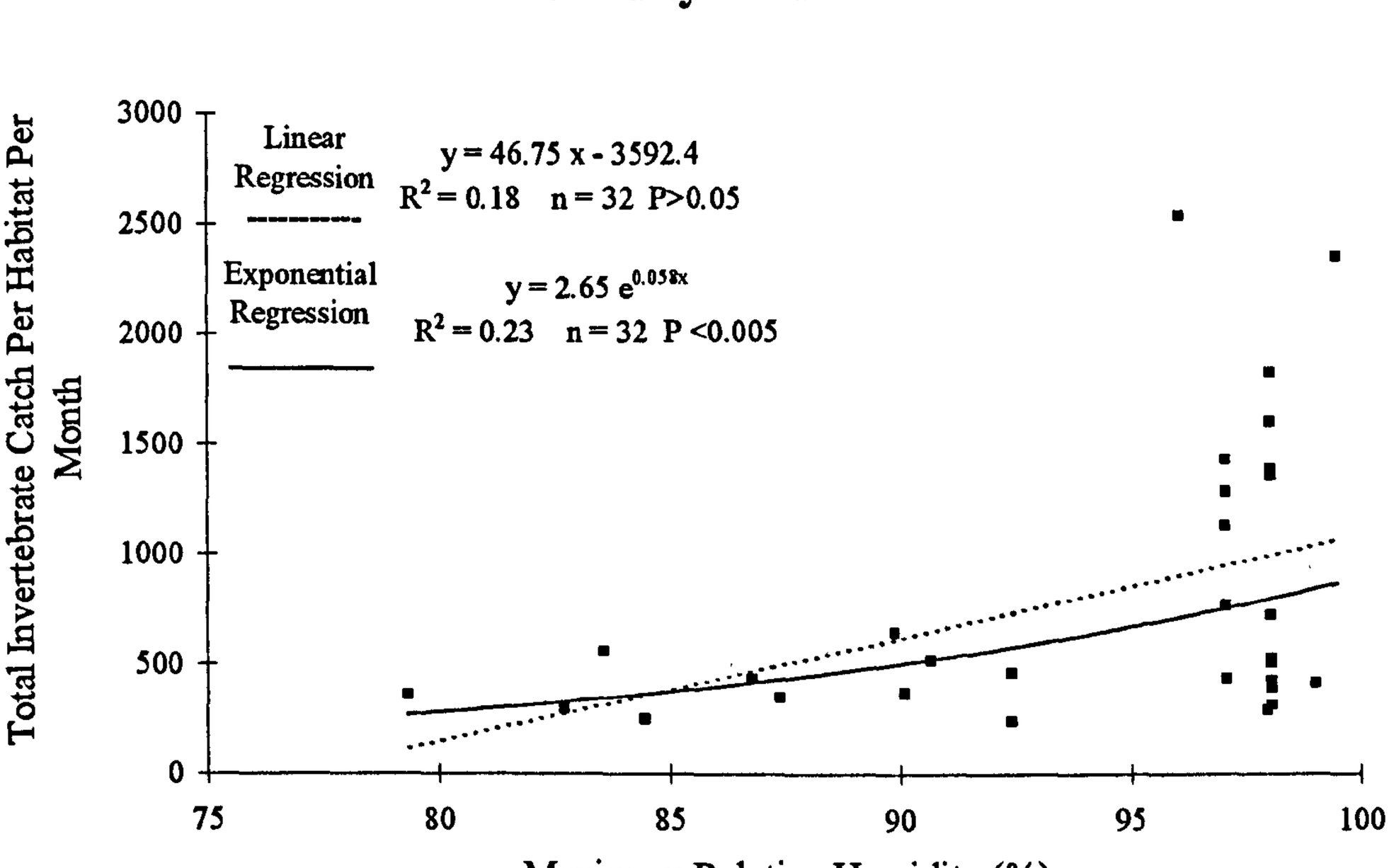


Figure 4.13



Four habitats x four months x two years = 32; the number of points used in the regression analysis.

4.5 DISCUSSION

Pitfall trapping potentially provided a quick and easy method of sampling the ground moving arthropods available to sit-and-wait vertebrate predators. Pitfall trap avoidance by different species was a potential source of error in this investigation as the number of avoiders may have differed between habitats (Cornish 1992). The behaviour of individuals when encountering the trap has been shown to influence the size distribution frequencies of invertebrates caught (Benest 1989a). Small species are closer to the ground, can sense the trap and have a lower speed and are therefore more likely to avoid a trap than larger faster moving species (e.g. Hayes 1970).

Only cursorial arthropods were successfully sampled by pitfall traps therefore phytophagous arthropods such as Dermaptera were probably under-sampled. Isopoda showed considerable pitfall trap avoidance behaviour (Lewis 1988); *Oniscus* and *Porcellio* species were observed in large numbers on walls surrounding one part of the radio-tracking enclosure and were probably under-sampled by the pitfall traps.

Suction sampling provided a second method to augment the pitfall trap data. The suction sampler was serviced in winter 1994 and a fault was discovered in the suction mechanism. The low number of vertebrates caught in this year (N=93) was probably a result of this fault. The inefficient sampling in 1994 may have resulted in sampling bias causing the unusual distribution of invertebrates and size classes in 1994. The suction sampler was repaired before the 1995 samples were taken.

Suction sampling is subject to sampling errors in addition to those caused by the mechanical failure in 1994. Richmond and Graham (1969) used a suction sampler to sample fleahoppers and found efficiency differed mainly with the taxa of invertebrate. Suction samples represent point samples in time and therefore only represent the invertebrates in each habitat on the sampling dates. These problems do not prohibit use of suction sampling in comparative studies between habitats but do prohibit absolute estimates of invertebrate population size (Henderson and Whitaker 1977).

Suction traps in the current study, as in other studies, caught less invertebrates in the larger size categories than pitfall traps (e.g. Cornish 1992, Henderson and Whitaker 1977). This may be due to the ability of larger taxa to resist the vacuum by clinging to vegetation (Southwood 1978) or it may be due to the bias of pitfall traps to large fast-moving cursorial arthropods such as Carabidae. Since the two methods show opposing bias in size of invertebrates caught, combination of the results from the two methods is assumed to improve the reliability of comparison of prey availability between habitats. Some taxa such as Oligochaeta will be undersampled by both methods of trapping. Oligochaeta may be able to avoid or escape from pitfall traps (Luff 1975) and Oligochaeta may be able to bury and therefore be undersampled by suction samplers. The percentage abundance of Oligochaeta in the current study was low (under 5%) however this may reflect a low abundance of Oligochaeta in the radio-tracking enclosure. Cornish (1992) caught Oligochaeta in 100% of pitfall traps using the same pitfall trap design used in the current study.

Habitat influenced the total numbers of invertebrates, percentage abundance of different taxa, size classes and dry mass of taxa caught by pitfall and suction traps. Different habitats provided different prey numbers, differences in the distributions of prey types (taxa) and differences in the biomass available to *B. bufo*. This emphasises the importance of monitoring food availability in a study of prey selection. The traps in woodland and rough grassland caught larger total numbers of invertebrates due to higher captures of small invertebrates (<4mm) than traps in arable and improved pasture. Both methods of trapping found a high percentage abundance of Acari in woodland and rough grassland despite the low percentage extraction of Acari by suction traps (Arnold *et al* 1973). The main constituent of Acari were Oribatid mites which are detritivores. Arable and improved pasture habitats lack a defined litter layer and therefore had a lower percentage abundance of Acari.

Collembola also contributed to the log normal distribution of size classes with a peak of larger Collembola (2-4mm) from nocturnal pitfall traps in arable during 1995 (Figure 4.7). Most species of Collembola are found in leaf litter feeding on decaying vegetable matter and fungi (Chinery 1986). Isopoda also had a significantly higher

percentage abundance in nocturnal traps (Figure 4.7) compared with diel traps probably explained by the fact that they are usually active at night (Hopkin 1991). Hemiptera were the third highly abundant small (<4mm) taxon in the traps. Arable in 1995 had a significantly larger number of invertebrates than arable in either of the previous two years probably due to an infestation of aphids (Hemiptera) on some of the crop plants. There was a greater percentage abundance of Opilones and Aranae in improved pasture than any other habitat (Figures 4.6 and 4.7). Duelli *et al* (1990) confirmed that pastures and short crop meadows had higher abundances of Aranae as they provided open areas for the hunting spiders.

Percentage abundance in middle size classes (8-14mm) differed between habitats largely due to the different contributions of Coleoptera (Figures 4.3 and 4.4). Arable and improved pasture had a higher percentage abundance of Coleoptera than other habitats (Figures 4.6 and 4.7). The dominance of Coleoptera and in particular Carabidae, in arable habitats has been demonstrated by many workers (e.g. Duelli *et al* 1990, Hance *et al* 1990, Liebman *et al* 1993). Pitfalls have a high rate of Carabidae capture as these beetles are active, ground moving predators and are therefore more likely to encounter traps than more sessile invertebrates such as Pulmonata (Greenslade 1963 and 1964, Baars 1979).

Pollard (1968) found that the peak occurrence of nocturnal species activity in arable habitats in July and August, coincided with the maximum crop cover. Luff (1978) showed that many species caught by pitfall traps were nocturnal, and peak numbers of Carabidae were caught at night. However in the current study there were no significant differences between nocturnal and diel pitfall trap percentage abundances of Coleoptera (Figures 4.6 and 4.7). Diel traps in the current study included both nocturnal and diurnal invertebrates therefore nocturnal and diurnal comparisons are not valid from the current data. In addition to the nocturnal sampling effort was lower than the diel sampling effort in terms of both numbers of traps and length of trapping period. Greenslade (1963) studied captive Carabidae from different habitats and found that the diel activity of the beetle depended on which habitat they were from due to different species distributions per habitat. In the current study there was a higher

percentage of Coleoptera, mainly Carabidae, in nocturnal traps from arable and improved pasture habitats than from other habitats (Figures 4.6 and 4.7). This data must be interpreted with caution due to the low sampling frequency (two pitfall traps per habitat).

Coleoptera made up >50% of the dry mass of taxa but were numerically the 2nd to the 5th largest in percentage abundance (Figures 4.6, 4.7 and 4.8). The high Coleopteran dry mass of pitfall catches in arable compared with other habitats was also linked to a higher abundance of predatory Coleoptera mainly Carabidae. The drop in dry mass between 1994 and 1995 was due to a reduction in the numbers of Coleoptera trapped in the latter year (Figures 4.6, 4.7 and 4.8). The drop in the percentage abundance of Coleoptera of both main and nocturnal traps in 1995 could have been due to climatic factors or to alterations in the habitats. In the Winter of 1994, habitat positions were altered by management practices described in chapter 3.

One of the most important habitat treatments was the creation of arable in the area formerly occupied by the long grassland. These practices are unlikely to have produced a reduction in Coleoptera numbers as Carabidae are mobile with good powers of dispersal and disturbance usually increases the number of Coleoptera found (Duelli *et al* 1990, Day *et al* 1993). The arable habitat in 1995 had a significantly higher number of smaller size class invertebrates than in arable in 1993 and 1994 (Figures 4.2, 4.3 and 4.4). This could be due to a carry over of small invertebrates from the former rough grassland (the arable in 1995). Carry over between the two years in this habitat is unlikely as the sod layer was removed and a deep sod layer is required for invertebrates to over-winter (Lagerlof and Wallin 1993).

The major difference in arable in 1995 compared with previous years was the large numbers of aphids which infested the crop plants, probably abundant due to prevailing weather conditions. The microclimate in the radio-tracking enclosure was significantly cooler and wetter in 1994 than in 1995. The microclimate analysis is discussed in chapter 6. Microclimate is also more likely to have influenced the decrease in Coleoptera from 1994 to 1995. Populations of Coleoptera are influenced by

microclimate (Hance and Gregoire-Wibo 1987) and total invertebrate numbers were significantly correlated with microclimate variables in the current study. Regressions though significant were relatively poor fits with a combined R^2 of less than 0.35. The number of invertebrates increased in a single step suggesting threshold temperatures for the activity of the taxa caught in the pitfall traps, resulting in the "all or nothing" response per taxon and a step function in the total invertebrate response to climate. Invertebrate numbers also showed an "all-or-nothing" response to maximum humidity.

4.6 CONCLUSIONS

- Invertebrate dry mass and percentage abundance differed between habitats and between years.

- Size class distributions were log normal with up to 80% of invertebrates in the smallest size class (<2mm). Size class distributions differed between habitats.

- Dominance of small taxa in terms of percentage abundance was a result of high numbers of Acari, Collembola and Hemiptera in diel traps.

- There were significantly more Acari in woodland and rough grassland than in arable and pasture, resulting in more small invertebrates in the former habitats.

- Dry mass was influenced most by Coleoptera which constituted >50% of total dry mass. Arable had a higher total dry mass than other habitats due to a higher percentage abundance of Coleoptera. Pitfall trap catches in 1995 had lower dry mass due to lower total numbers of Coleoptera compared with 1994.

5: DIET AND PREY SELECTION

5.1 INTRODUCTION

The diets of small vertebrate insectivores (arthropodivores) have been described as opportunistic or generalist (e.g. Klimstra and Meyers 1965). An opportunist or generalist will eat whatever it encounters and will take prey in proportion to those in the environment. In studies where both prey availability and diet have been measured then small vertebrate insectivores demonstrated diet specialisation to varying degrees (Loman 1979, Jaeger and Bernard 1981, Toft 1981, 1985).

The anurans are some of the most widespread small vertebrate predators in the United Kingdom. *B. bufo* are opportunistic feeders and toad stomach contents have reflected the dominant ground moving arthropods in a foraging area, such as coleopterans and hymenopterans (Semb-Johansson 1989, Denton 1991, Cornish 1992). *B. bufo* have even been recorded feeding on amphipods and marine organisms at low tide (Semb-Johansson 1989). Coleopterans and hymenopterans are the most commonly found taxa in 14 species of bufo throughout the world (Clarke 1974). The diet of *R. temporaria* also contains large numbers of coleopterans (Kminiak 1978, Blackith and Speight 1974). The high proportion of coleopterans in anuran diets has lead some workers to suggest their use for biological control of crop pests (Harding 1972, Karg and Mazur 1969, Atatur *et al* 1993).

The wide range of taxa in the diet of *B. bufo* has lead many workers to suggest that they are unselective (Lescure 1964). *B. bufo* are not completely unselective as they will select fast moving prey in preference to slow moving organisms (Larsen and Pedersen 1982). Prey selection is also a function of size, with smaller and juvenile *B. bufo* restricted to smaller prey items by the physical constraints of gape width (Kuzim 1990, Cornish 1992). Taxa eaten by juvenile *B. bufo*, like those in the diets of adults, are dominated by ground moving invertebrates especially Acari, Collembola, Formicidae and small Coleoptera (Lescure 1964, Harding 1972). The restraints of gape width may segregate prey selection between age classes and reduce competition (Harding 1972, Wheater 1986, Gittins 1987).

B. bufo are nocturnal predators and adult *B. bufo* can actively forage (Lescure 1964) but most research suggests a 'sit-and-wait' or ambush strategy is usually adopted. An ambush strategy minimises moisture loss, energy expenditure and risk of predation (Clarke 1974, Klimstra and Myers 1965, Duellman and Trueb 1986, Swan 1986). Bufonids locate their food using mainly visual cues, with olfactory cues playing only a reinforcing role in some species, even at low light intensities (Larsen and Pedersen 1982). Once prey is detected, the tongue is flipped out then retracted and prey is drawn down into the buccal cavity as a mechanical response elicited by the presence of an object in the mouth (Larsen 1984, Duellman and Trueb 1986).

The foraging behaviour and diet of recently metamorphosed toadlets (metamorphs) is not well understood. Prey taxa of metamorphs consisted of Aranae, Acari, Collembolla and Staphylinidae (Cornish 1992). Analysis of diet and observation of metamorphs suggested they actively forage (Harding 1972, Larsen 1984). The segregation of foraging behaviours between adult sit-and-wait and juvenile active foragers was observed by Clark (1974) in *Bufo woodhousei fowleri*.

5.2 APPROACH AND AIMS

The experiments described in this chapter determined the prey eaten by male and female *B. bufo*. The relationship between foraging and habitat preference was assessed by comparing of diet of *B. bufo* with prey availability in different habitats. Data on prey availability are provided in chapter 4. Diet was assessed in terms of percentage abundance and dry mass of different taxa in the stomachs of *B. bufo* from different habitats.

5.3 METHODS

5.3.1 Collection Of Samples

At the end of their tracking periods (chapter 3) *B. bufo* were made to regurgitate their stomach contents by palpation. Termination of tracking often occurred during periods of inactivity so most stomach contents of tracked *B. bufo* were empty or heavily digested. Many *B. bufo* were lost for reasons described in chapter 3 before their

stomach contents could be collected. This resulted in the collection of only ten toad stomach contents in both 1993 and a further ten in 1994.

During April - September 1995 a circuit around the radio-tracking enclosure and the nearby breeding pond was traversed by Dr R.S. Oldham and the stomach contents of any *B. bufo* found were collected by palpation. The habitat in which the *B. bufo* were located was recorded. When the habitat was homogenous *B. bufo* were assumed to have been feeding in these habitats (69 stomachs). If habitat was heterogeneous then habitat was recorded as unclassified (39 stomachs). The dorsal lengths of *B. bufo* were measured (from the tip of the snout to the posterior end of the urostyle) to the nearest mm using an angled ruler and the mass in g recorded, on a field balance, to the nearest mg.

5.3.2 Identification And Classification Of Diet

Five cm^3 of ethanol was placed into a $10cm^3$ measuring cylinder, the fresh stomach contents (boluses) were placed into the ethanol. Taxa were identified as described in chapter 4. Some boluses consisted of body parts of prey organisms, these were assembled into individual organisms if possible, if not possible "marker segments" were counted, for example beetles can only have one pair of elytra and these were usually the last things to digest. In Dermaptera the tail pincers remained undigested, and ant heads were counted. Once identified and counted all contents were dried in air for 24 hours, then dried at 60°C for 24 hours. The dry mass of each taxon was recorded to 4 decimal places and the total dry mass was calculated as the sum of all taxa dry masses. Dry mass and direct counts of prey taxa were chosen as these provide direct measurements of quantity of prey. Size of prey items can be estimated indirectly by measuring marker segments and converting this to dry mass via predetermined indices (e.g. Sage 1960).

If *B. bufo* were captured soon after feeding, then stomach contents were very undigested and the capture habitat was more likely to be the feeding habitat. Stomach content samples are biased in favour of hard bodied organisms such as coleopterans, due to their slow digestion time (Houston 1973). Since taxa differ in their rates of

digestion, less digested stomach contents will reflect the diet more accurately. An index of digestion was therefore calculated for each stomach content analysed. Data from Whittaker (1995) and from personal observation were used to rank decomposition of individual taxa. The decomposition table for Coleoptera is included as an example (Table 5.1) all other tables are in Appendix C. Digestion tables for Coleoptera, Pulmonata, Isopoda, and Oligochaeta (Appendix C) are based on experiments done by Whittaker (1995). *B. bufo* were fed a set number of the chosen taxon and stomach contents were regurgitated at set time intervals, descriptions of digestion were made and digestion was ranked from 1 (very undigested) to 5 (digested) using identification points (Table 5.1).

Table 5.1

Digestion Of Coleoptera By *B. bufo*

DIGESTION RANK	IDENTIFICATION POINT	GENERAL DESCRIPTION	TIME
1	No joint movement	The animals are still whole with almost no decomposition. The leg joints remain firm and the antennae and legs are mostly present	0 hours +
2	Leg joints loose but still attached	The leg joints become flexible and loose but most legs are still attached and the body is still whole. The antennae may be missing but the body joints are firm and attached	9 hours +
3	The leg joints loosen or detach from body. The body joints become loose	The body is still whole but the joints become loose and the legs become detached. Some legs and antennae may be missing. The leg joints are loose	16 hours +
4	Body begins to separate into component parts	The body joints split at abdomen head and thorax. Some of the abdominal segments separate. The legs are missing or detached and the leg joints are loose or separated.	19 hours +
5	All segments are separated.	All the joints have broken down and the elytra wings and abdominal segments are separated. Many pieces may be missing.	22 hours - 48 hours

Data are based on Whittaker 1995. Identification points were used to assign a digestion rank to each item found in the stomachs of *B. bufo*.

Using the Whittaker work as an example, 20 stomachs from unclassified habitats were used to draw up digestion tables for the following taxa: coleopteran larvae, Diptera, Dermaptera, Formicidae, Diplopoda, Formicidae and Homoptera (aphids). All other stomach contents were then identified and each item found was given a rank of 1 to 5 for digestion using the tables. Digestion ranks for taxa with no digestion table were estimated using the table for the most morpholgically similar taxon. For example, digestion ranks of Chilopoda were estimated using the table for Diplopoda, ranks for lepidopteran and dipteran larvae were estimated from the table for coleopteran larvae. Where no morphological equivalent was found no digestion rank was assigned. The mean digestion rank of each bolus was calculated by dividing the sum of the digestion ranks per taxon by the total number of taxa for which a rank was assigned.

5.3.3 Analysis

The boluses collected in 1995 were ranked into those from *B. bufo* caught in arable or pasture and those from woodland or long grassland habitats. The habitats were grouped as numbers of *B. bufo* of each sex in the four separate habitats were low (<10). Radio-tracked *B. bufo* given a choice of habitats preferred woodland and rough grassland (Chapter 3) therefore boluses from *B. bufo* caught in these habitats were grouped together. Arable and pasture were the least preferred habitats by radio-tracked *B. bufo* and boluses from *B. bufo* caught in these two habitats were grouped. If the habitats were not known the data were assigned to a third class called unclassified.

To asses if the toad size (length and mass) varied with sex, the length and mass of *B. bufo* were used in a Two-way MANOVA with sex of individual and capture habitat as independent variables. To determine whether larger *B. bufo* had a higher mass in stomachs the total dry mass of boluses was regressed against toad mass and length after statistical outliers were removed. The total dry mass of boluses of males and females from different habitats were compared by a two-way ANOVA. To asses if prey taxa varied with sex, the percentage abundance of taxa in the diets of males and females were compared in a two-way MANOVA with habitat and sex as the

independent variables. A second MANOVA, with the same independent variables, was used to compare the dry mass of taxa. An index of digestion was calculated as 1/mean digestion rank of each bolus. The digestion index was used to weight the MANOVAs of 1995 data using the "by case weight" command in SPSS (Norusis 1988).

All stomachs from the three years were used to calculate a niche breadth using Levins (1968) niche breadth B, $B = 1/\sum Pi^2$

Pi is the percentage of the total diet made up by the i^{th} item. Shannon-Wiener niche diversity (Shannon 1948) H' was also calculated

$$H' = -\sum_{i=1}^{s} Pi \ln Pi$$

P_i is the proportion of prey item i in diet . Evenness J' was calculated using Krebs (1989)

$$J' = \frac{H'}{\ln S}$$

H' is the niche diversity calculated as above and S is the total number of food items ascertained. The percentage taxa in the diet of *B. bufo* caught within the radio tracking enclosure were compared with those of the *B. bufo* caught outside the enclosure, using a one-way MANOVA.

Data from chapter 4 were used to provide the prey availability data for the following calculations. Prey availability in the current study refers to the prey caught by suction and pitfall traps. In each year the number of each prey taxon in all *B. bufo*' stomachs were summed and then converted to percentage of the total number of prey items eaten in that year to produce yearly percentage of each taxon in the diet. The number of each taxon in diel pitfalls from all habitats and the number of each taxon from suction traps were summed and divided by the total prey items caught each year to produce yearly diel prey availability. The diet of *B. bufo* and the diel prey availability were compared for each year using the diel electivity index D (Jacobs 1974)

$$D = -\frac{d_k - f_k}{d_k f_k - 2 d_k f_k}$$

where d_k is the proportion of prey k in the stomach contents and f_k is the proportion of prey k in the environment. D varies from +1 for complete selection or preference of prey k, through 0 when prey k is taken in the same proportion as found in the environment, to -1 when prey k is absent in the diet but present in the environment. The calculation was repeated using the data from the nocturnal pitfall traps and the suction trap data to produce nocturnal prey availability and nocturnal electivity index.

5.4 RESULTS

A total of 20 toad stomach contents were obtained from the radio-tracked *B. bufo* (chapter 3). Stomach contents from 64 females and 44 males were obtained in 1995 from the local breeding population outside the radio-tracking enclosure. The lengths and masses of *B. bufo* from outside the radio-tracking enclosure are given in Figures 5.1 (a) and (b).

Toad size (mass and length) varied with sex (Hotellings $T^2_{2,53} = 0.35$, $P<0.0001$) but not with habitat (Hotellings $T^2_{2,53} = 0.03$, $P>0.05$) and there was no interaction (Hotellings $T^2_{2,53} = 0.07$, $P>0.05$). Mean mass of females was 25.1g (+/-1.5g) and mean length was 58.4mm (+/-1.3mm). The males were smaller than the females with a mean mass of 17.1g (+/-1.0g) and mean length was 53.7mm (+/-0.7mm). Digestion values of boluses varied from 1.30 to 4.20 with a mean of 2.75 (+/-0.6).

5.4.1 Dry Mass

There was no significant correlation between toad mass and total dry mass of boluses ($R^2= 0.071$, $F_{1,98} =6.79$, $P>0.05$). There was a weak but significant correlation of total dry mass of boluses with toad length ($R^2= 0.108$, $F_{1,98} =8.77$, $P<0.005$). The regression equation and r^2 value for the significant correlation are given in Figure 5.2.

The dry masses of individual taxa in the diet of *B. bufo* differed with capture habitats (Hotellings $T^2_{13,219} =0.365$, $P<0.001$) and with sex (Hotellings $T^2_{13,219} =0.168$, $P<0.005$) and the two factors interacted (Hotellings $T^2_{13,219} =0.303$, $P<0.001$).

Figure 5.1

Mean Mass And Length of *B. bufo*

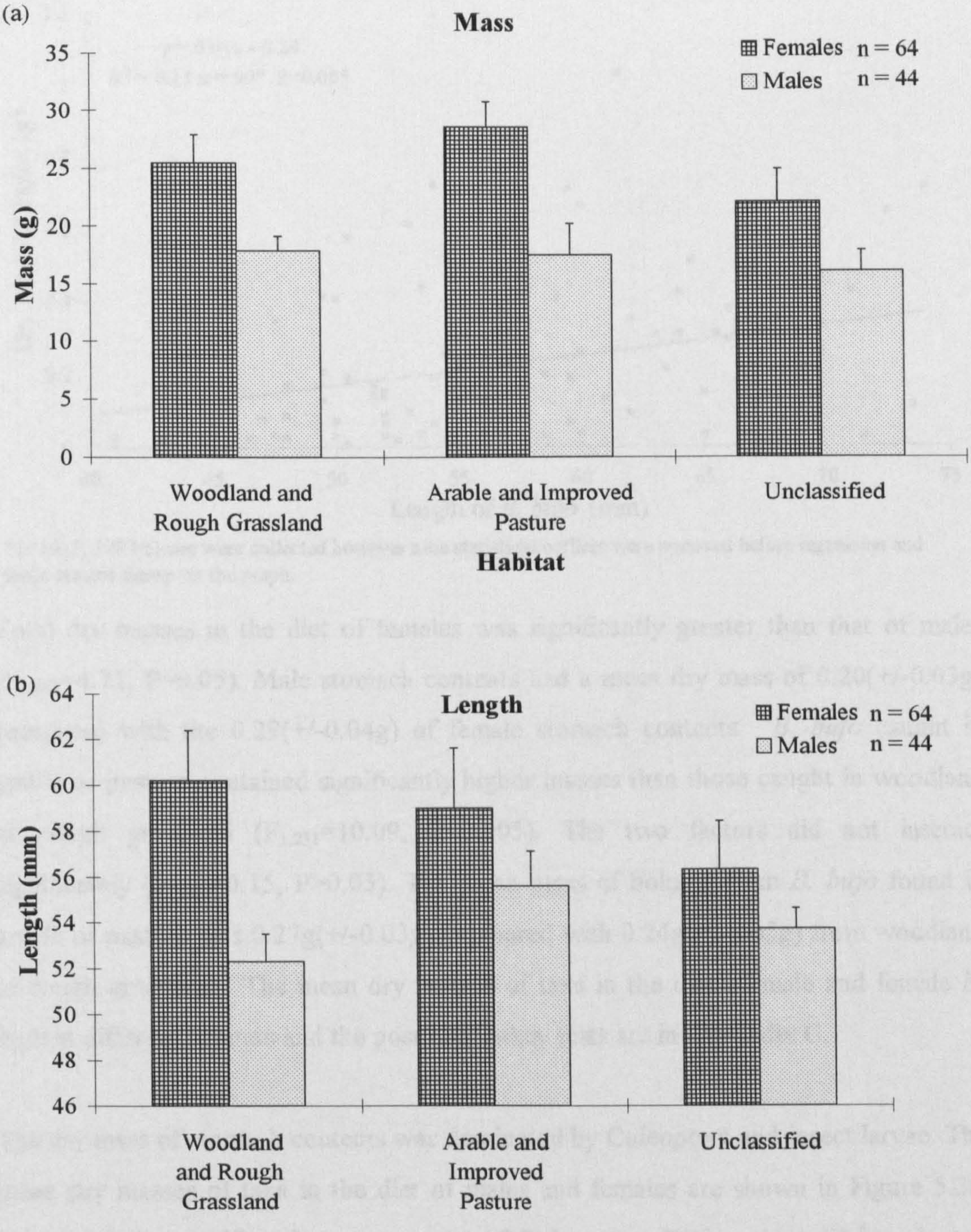
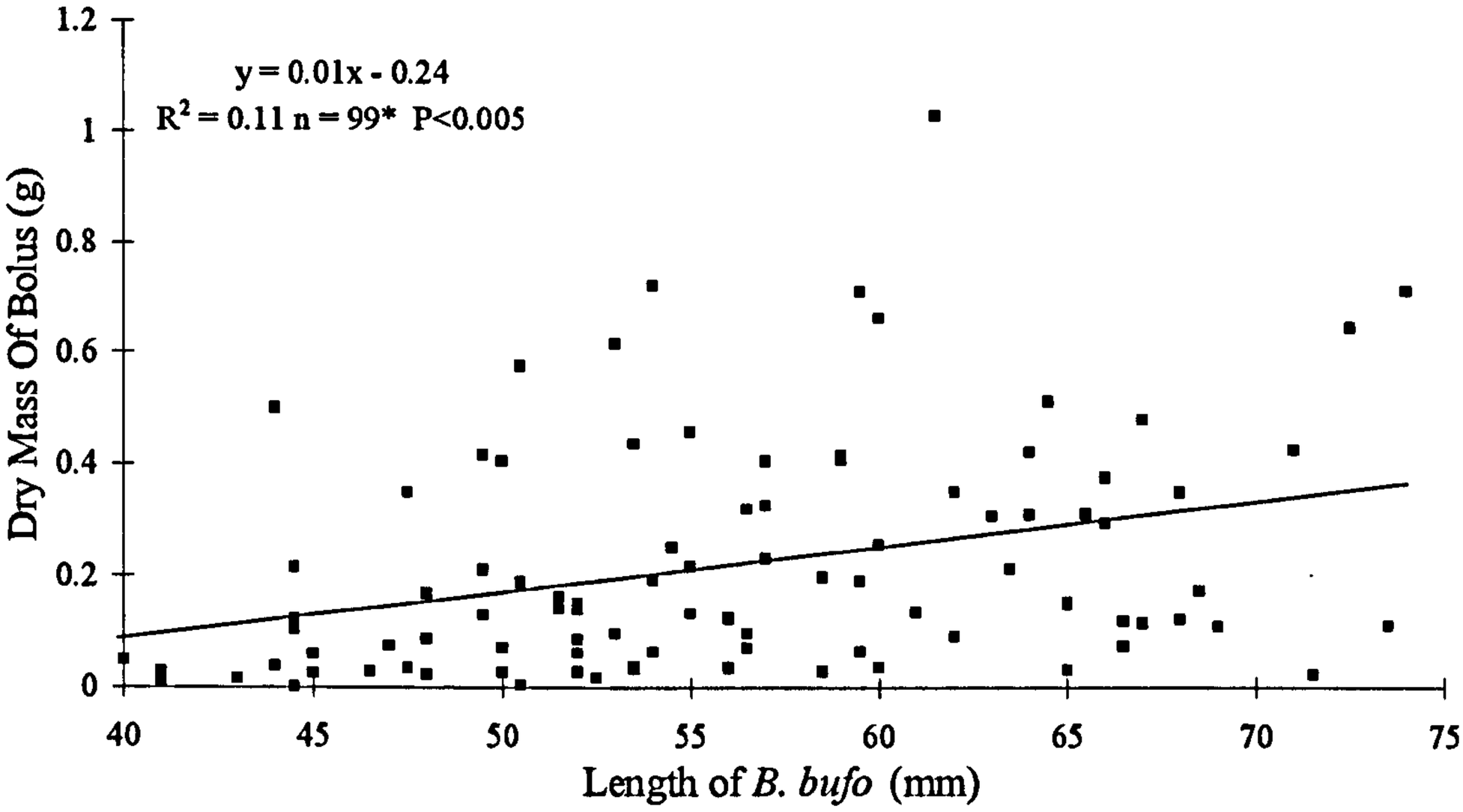


Figure 5.2

Dry Mass Of Bolus Of Stomach Contents And Length Of *B. bufo*

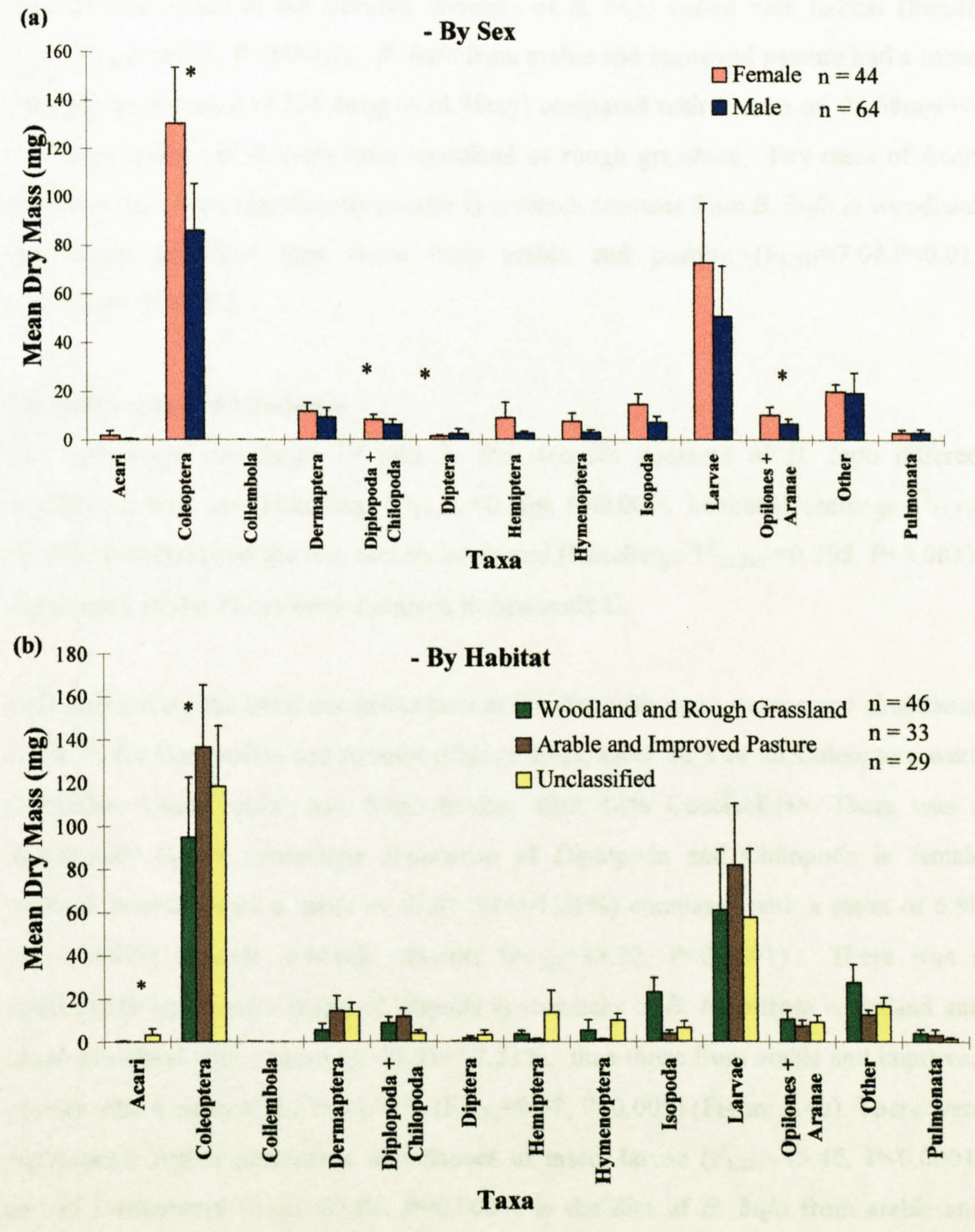


*In 1995, 108 boluses were collected however nine statistical outliers were removed before regression and these are not shown on the graph.

Total dry masses in the diet of females was significantly greater than that of males ($F_{1,231}$=4.71, P=0.05). Male stomach contents had a mean dry mass of 0.20(+/-0.03g) compared with the 0.29(+/-0.04g) of female stomach contents. *B. bufo* caught in arable or pasture contained significantly higher masses than those caught in woodland or rough grassland ($F_{1,231}$=10.09, P<0.005). The two factors did not interact significantly ($F_{1,231}$=0.15, P>0.05). The mean mass of boluses from *B. bufo* found in arable or pasture was 0.27g(+/-0.03g) compared with 0.24g(+/-0.05g) from woodland or rough grassland. The mean dry masses of taxa in the diet of male and female *B. bufo* in different habitats and the post hoc Tukey tests are in Appendix C.

The dry mass of stomach contents was dominated by Coleoptera and insect larvae. The mean dry masses of taxa in the diet of males and females are shown in Figure 5.3a. Females had a significantly greater mass of Coleoptera, Diplopoda + Chilopoda and Opilones + Aranae than males ($F_{1,231}$=7.01,P<0.01; $F_{1,231}$=6.79, P<0.01; $F_{1,231}$=8.82, P<0.005 respectively). Females had a mean of 130.2mg(+/-23.26mg) of Coleoptera in stomach contents compared with only 85.84mg(+/-19.25mg) for males. Males contained significantly greater mass of Diptera than females ($F_{1,231}$=9.67, P<0.005)

Figure 5.3



Data are from 128 *B. bufo* sampled. Bars are +/- the standard error of the mean. Other = all other invertebrate taxa in stomach contents including Oligochaeta. Larvae = insect larvae, mostly coleopteran, dipteran and lepidopteran larvae. * represent significant between habitat /sex differences in the dry mass of a taxon. n = the number of *B. bufo* from each category used in dietary analysis.

Dry mass in both habitat types were dominated by Coleoptera and insect larvae with their combined mass greater than 69% of total dry mass of stomach contents. Dry mass of Coleoptera in the stomach contents of *B. bufo* varied with habitat (Figure 5.3b) ($F_{1,231}$=20.75, P<0.0001). *B. bufo* from arable and improved pasture had a mean coleopteran dry mass of 136.4mg(+/-28.95mg) compared with a mean of 94.68mg(+/-27.77mg) in diets of *B. bufo* from woodland or rough grassland. Dry mass of Acari and other taxa were significantly greater in stomach contents from *B. bufo* in woodland and rough grassland than those from arable and pasture ($F_{1,231}$=7.04,P<0.01; $F_{1,231}$=4.69, P<0.05).

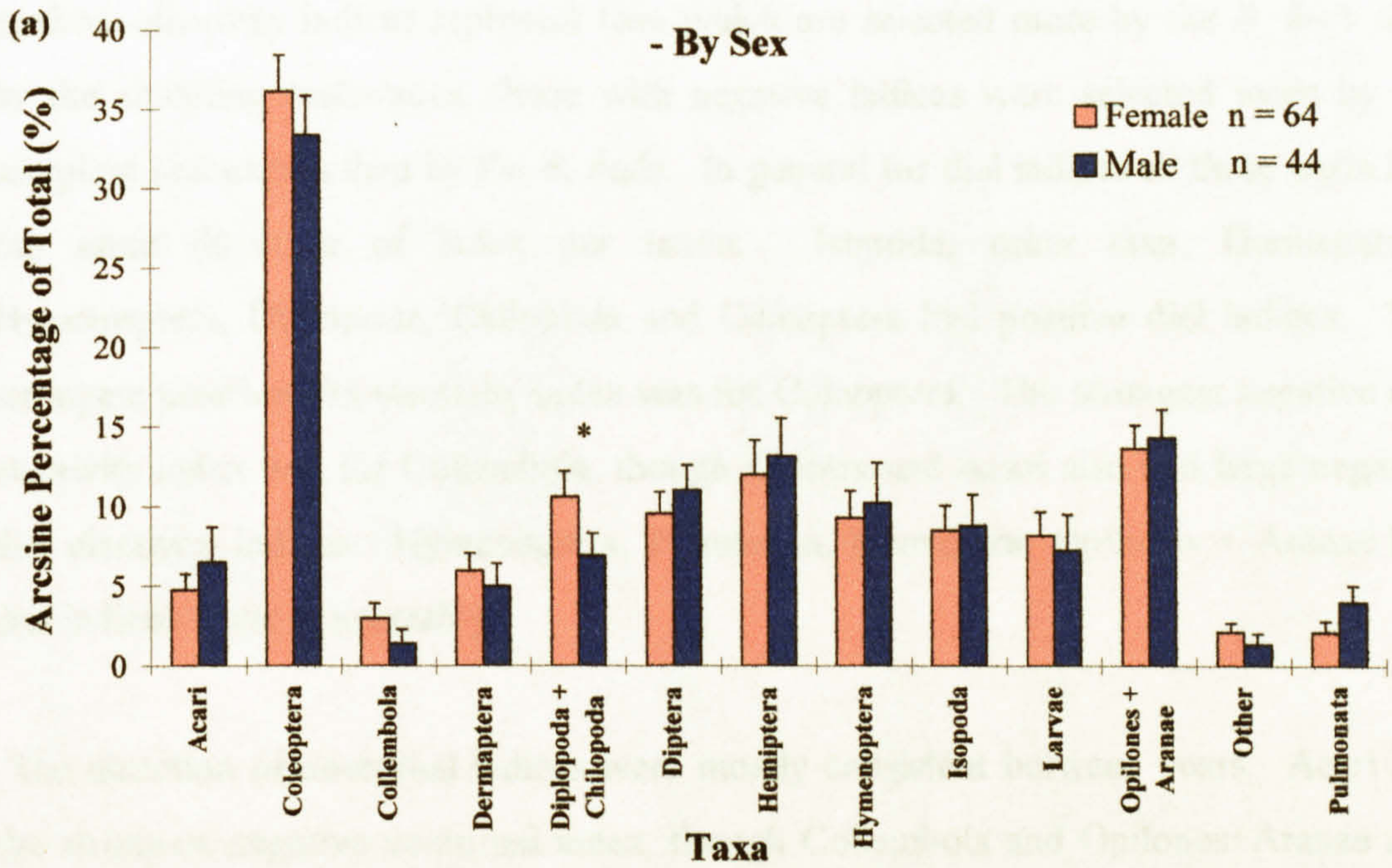
5.4.2 Percentage Abundance

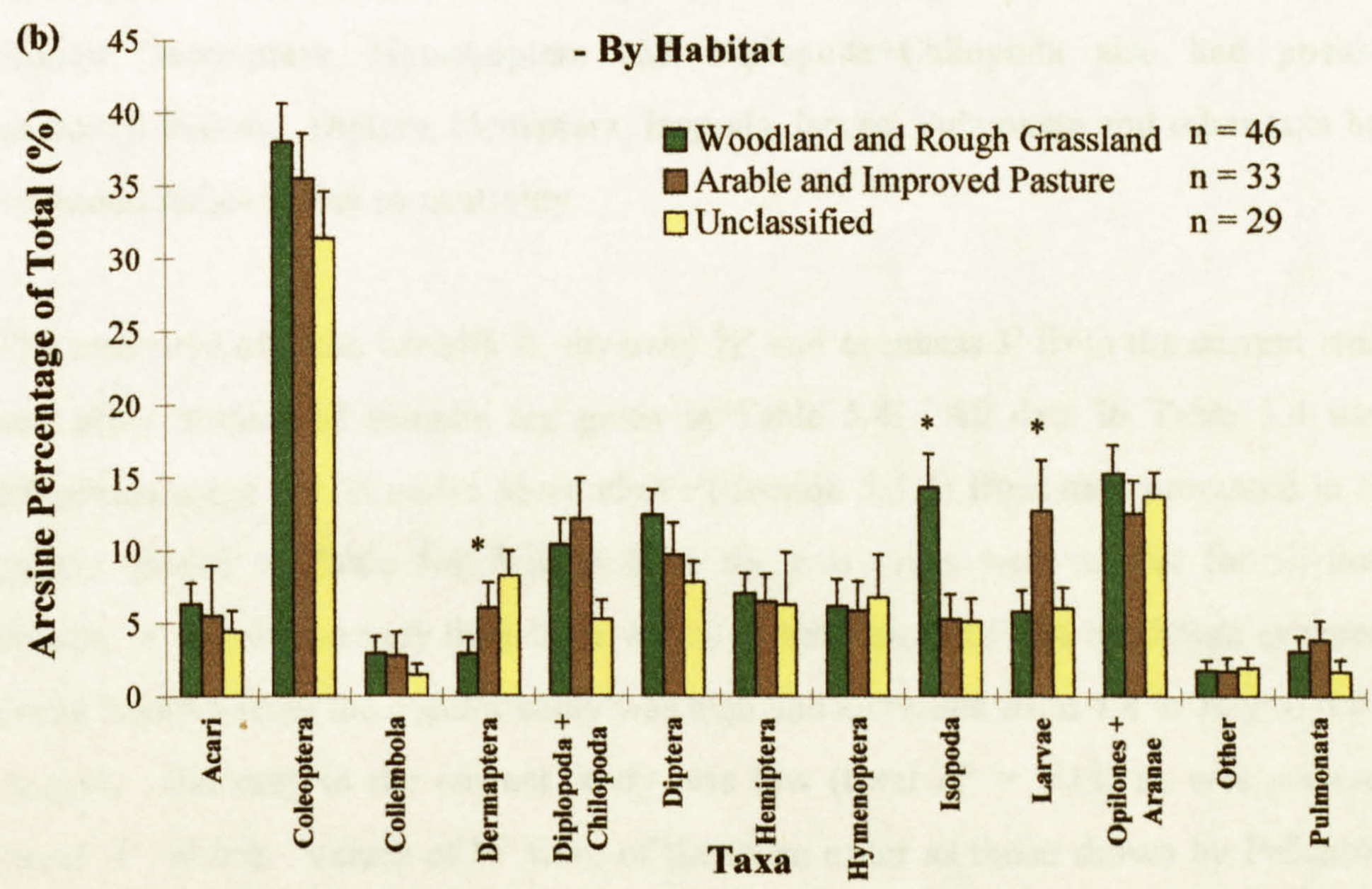
The percentage abundance of taxa in the stomach contents of *B. bufo* differed significantly with sex (Hotellings $T^2_{13,219}$ =0.169, P<0.005) habitat (Hotellings $T^2_{13,219}$ =0.400, P<0.001) and the two factors interacted (Hotellings $T^2_{13,219}$ =0.398, P<0.001). A summary of the Tukey tests are given in Appendix C.

Coleoptera were the most numerous taxa in the diet with mean percentage abundance of >30% for both males and females (Figure 5.4a). Over 80% of all Coleoptera were Carabidae, Curculonidae and Staphylinidae, with 12% Coccinelidae. There was a significantly higher percentage abundance of Diplopoda and Chilopoda in female stomach contents with a mean of 10.62 %(+/-1.61%) compared with a mean of 6.90 %(+/-1.46%) in male stomach contents ($F_{1,231}$=13.22, P<0.0001). There was a significantly higher percentage of Isopoda in stomachs of *B. bufo* from woodland and rough grassland with a mean of 14.31+/-2.31%, than those from arable and improved pasture with a mean of 5.27+/-1.72% ($F_{1,231}$=9.07, P<0.005) (Figure 5.4b). There were significantly higher percentage abundances of insect larvae ($F_{1,231}$=15.46, P<0.0001) and of Dermaptera ($F_{1,231}$=23.84, P<0.0001) in the diet of *B. bufo* from arable and improved pasture than those from woodland and rough grassland.

Figure 5.4

Mean Percentage Abundance Of Taxa In The Diet Of *B. bufo*





Data are from 128 *B. bufo* sampled. Bars are +/- the standard error of the mean. Other = all other invertebrate taxa in stomach contents including Oligochaeta. Larvae = insect larvae, mostly coleopteran, dipteran and lepidopteran larvae. * represent significant between habitat or between sex differences in the dry mass of a taxon. n = the number of *B. bufo* from each category used in dietary analysis.

5.4.3 Electivity, Niche Breadth And Evenness.

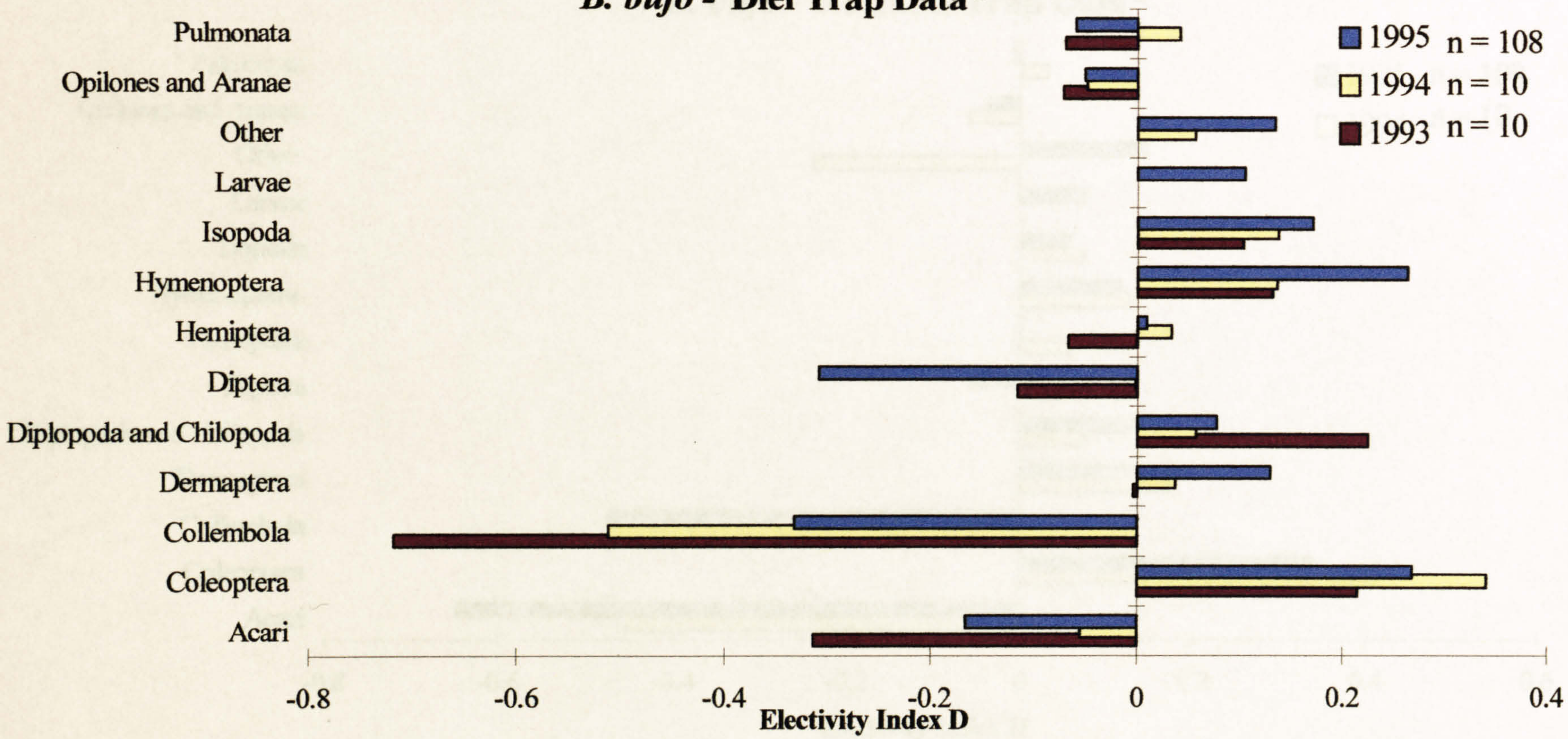
The diel and nocturnal electivity indices of different taxa are shown in Figures 5.5 and 5.6 respectively, the electivity index values are given in Table 5 of Appendix C. The positive electivity indices represent taxa which are selected more by the *B. bufo* than by the sampling techniques, those with negative indices were selected more by the sampling techniques than by the *B. bufo*. In general for diel indices all three years had the same direction of index per taxon. Isopoda, other taxa, Dermepatera, Hymenoptera, Diplopoda, Chilopoda and Coleoptera had positive diel indices. The strongest positive diel electivity index was for Coleoptera. The strongest negative diel electivity index was for Collembola, though Diptera and Acari also had large negative diel electivity indices. Hymenoptera, Pulmonata, Hemiptera, Opilones + Aranae had diel indices close to neutrality.

The direction of nocturnal indices were mostly consistent between years. Acari had the strongest negative nocturnal index, though Collembola and Opilones+Aranae also had negative nocturnal indices. Coleoptera had the strongest positive nocturnal index, though Dermaptera, Hymenoptera and Diplopoda+Chilopoda also had positive nocturnal indices. Diptera, Hemiptera, Isopoda, larvae, Pulmonata and other taxa had nocturnal indices close to neutrality.

The measures of niche breadth B, diversity H' and evenness J' from the current study and other studies of anurans are given in Table 5.4. All data in Table 5.4 were calculated using the formulae given above (Section 5.3.3) from data presented in the papers quoted in Table 5.4. Values from the two sexes were similar for all three indices. J' values can vary from 0 - 1 with 0 as minimum and 1 as maximum evenness. Niche breadth from the current study was high and increased from 4.4 in July to 6.8 in August. Diversity in the current study was low (total H' = 2.14) as was evenness (total J' =0.26). Values of H' were of the same order as those shown by Pellantova (1978) for the same species. Values of J were approximately half those given by Pellantova for the same species.

Figure 5.5

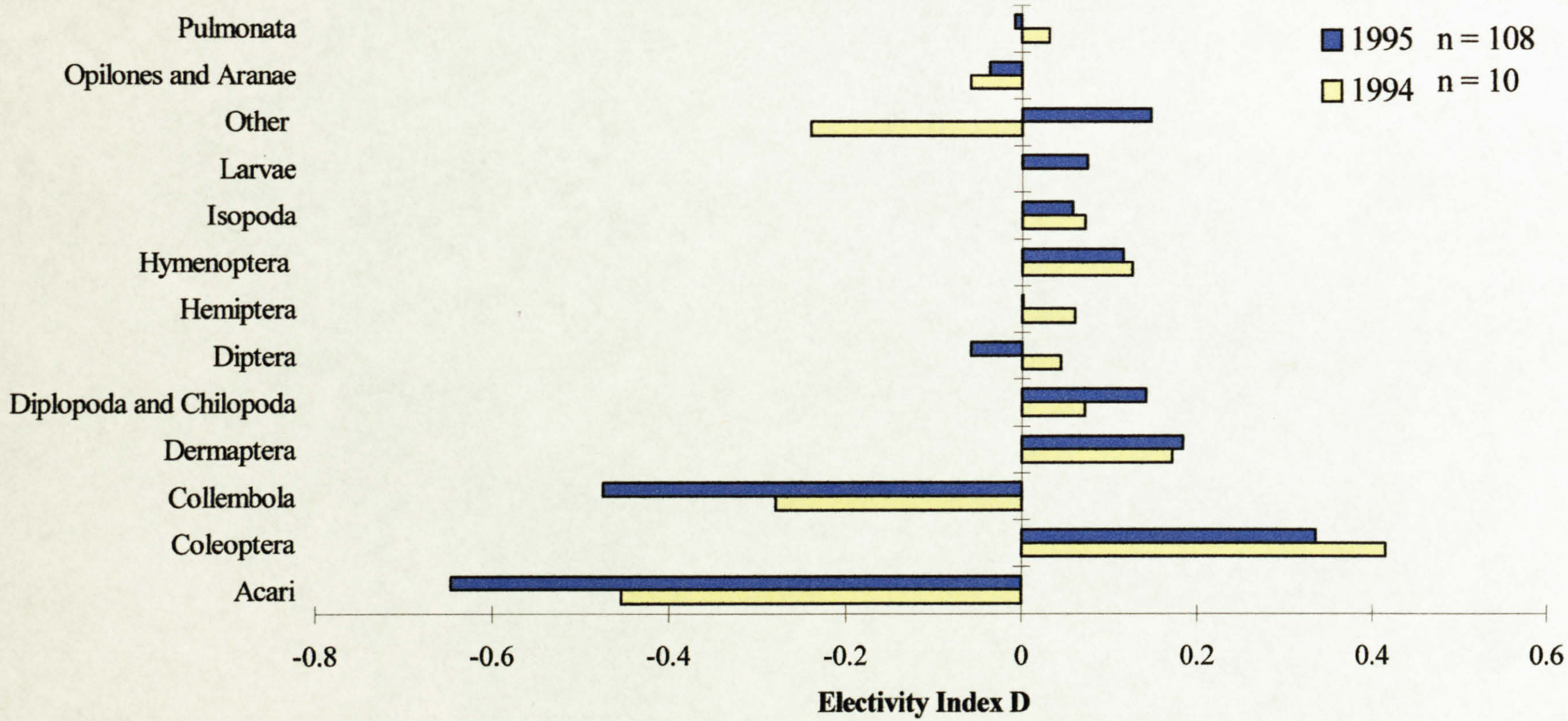
Electivity Indices Of Taxa In The Diet Of *B. bufo* - Diel Trap Data*



Calculation of Index D is described in the text. Selected taxa have values of D>0, values <0 are taxa with high percentage abundance in the environment but low abundance in the diet. Other = all other invertebrates in the diet of *B. bufo*. Larvae = insect larvae mainly coleopteran, dipteran and lepidopteran larvae.
* Percentage abundance of available taxa were calculated by combining suction trap data with **diel** pitfall trap data. n = the number of *B. bufo* used in dietary analysis in each respective year.

Figure 5.6

Electivity Indices Of Taxa In The Diet Of *B. bufo* - Noctural Trap Data*



Calculation of Index D is described in the text. Selected taxa have values of D>0, values <0 are taxa with high percentage abundance in the environment but low abundance in the diet. Other = all other invertebrates in the diet of *B. bufo*. Larvae = insect larvae mainly coleopteran, dipteran and lepidopteran larvae.
* Percentage abundance of available taxa were calculated by combining suction trap data with **nocturnal** pitfall trap data. n = the number of *B. bufo* used in dietary analysis in each respective year.

Table 5.4

Niche Breadth, Evenness and Diversity Indices

Species	Reference	NOTES	B	H'	J'
B. bufo	Current Study	Total	6.73	2.14	0.26
		Males	6.51	2.09	0.30
		Females	6.33	2.10	0.27
		July	4.40	1.84	0.25
		August	6.78	2.18	0.35
		September	6.80	2.15	0.26
	Pellantova 1978	Total	5.86	2.00	0.45
		March	4.51	1.55	0.44
		April	4.23	1.27	0.36
		June	4.22	1.70	0.68
		July	6.11	2.40	0.88
		September	3.29	1.28	0.66
Ceratophrys cornuta	Duellman and Lizana 1994	November	2.99	2.28	0.63
		December	6.71	3.14	0.82
		January	1.14	1.23	0.47
		February	2.24	1.94	0.58
		Other	2.27	1.81	0.64

B = niche breadth H' = diversity J' = evenness

Ceratophrys cornuta (horned frogs) are sit-and-wait strategists which eat large mobile arthropods and small vertebrates (Duellman and Lizana 1994). They have low prey niche Breadth because a low diversity of taxa are taken, evenness is high in *C. cornuta* as large numbers of each prey group are eaten. 72 individual frogs were examined, 11 stomachs were empty with a total of 507 prey items

The Pellantova (1978) study was conducted in southern Czech Republic. He analysed a total of 192 stomach contents over 55% of which were empty. A total of only 544 invertebrates were caught by his study

In the current study a total of 108 toad stomachs were collected in 1995 (empty stomachs were not recorded) containing a total of 3458 prey items.

5.5 DISCUSSION

Where stomach contents are used to quantify diet a measure of the rate of digestion is essential. Digestion of prey by *B. bufo* varies between prey taxa (Larsen 1984). Soft bodied organisms would be under-represented in terms of dry mass and percentage abundance in highly digested boluses, therefore the information obtained from these boluses could be described as less accurate. Using a weighting system is a mathematical way of interpreting data where accuracy of recording may vary between data points. On a subjective scale of 1 to 5 (Appendix C) the stomach contents in the current study had a wide range of digestion indices from 1.3 to 4.2, emphasising the importance of weighting the results of dietary analysis

5.5.1 The Diet Of *B. bufo*

Values of diversity (H') were similar to those given by Pellantova (1978) but niche breadth (B') from the current study were higher and evenness (J') were lower than those recorded by Pellantova (Table 5.4). Since J' is a function of H' and S (total prey numbers), variations in J between the two studies resulted from higher S values in the current study than in the Pellantova (1978) study (3458:544 respectively). In the current study niche breadth (B') was high but diversity (H') was low, because evenness (J') was low (Table 5.4). This was a result of a diet dominated by a limited number of taxa but including small numbers of a wide range of taxa. The dominant taxa in terms of dry mass and percentage abundance was Coleoptera.

Coleoptera, Opilones + Aranae, Hemiptera, Diplopoda + Chilopoda, Diptera and Hymenoptera; all had abundances of greater than 10% (Figure 5.4). This distribution pattern of prey taxa has been found in most studies of the diet of *B. bufo* (Semb-Johansson 1989, Denton 1991, Cornish 1992). Coleoptera and Hymenoptera have been recorded as the most abundant taxa in diets of many anurans including *R. temporaria* (Blackith and Speight 1974, Kminiak 1978, Toft 1981, 1985).

The contribution of different taxa to dietary mass is more important to *B. bufo* growth and condition than is their abundance. Coleoptera adults and all insect larvae contributed more than 69% of the dry mass in stomach contents of *B. bufo*. The

importance of Coleoptera has been confirmed in many studies (e.g.Lescure 1964, Larsen 1984). The importance of insect larvae has been overlooked for two main reasons; firstly the larvae are soft bodied and digest very quickly, secondly most studies concentrate on percentage abundance and not dry mass. In the current study larvae had low percentage abundance and high dry mass. Length of prey is related to it's calorific value (Schoener 1961) so large soft-bodied organisms with high nutritional value such as Lepidoptera larvae will be undervalued in studies of diets based on percentage abundance.

Diets differed with capture habitat; *B. bufo* from arable and pasture habitats had a significantly higher dry mass of Coleoptera and a lower mass of Acari than those from woodland and rough grassland (Figure 5.3b). *B. bufo* from the arable and pasture had a higher percentage abundance of larvae and of Dermaptera and a lower abundance of Isopoda than *B. bufo* from woodland and rough grassland (Figure 5.4b). These habitat specific differences in diet were a reflection of differences in food caught in traps in the respective habitats. For example arable and improved grassland habitats had higher dry mass and percentage abundance of Coleoptera than woodland and rough grassland (Figure 4.10). Change in diet with prey availability reflects a plasticity in the diet requirements of *B. bufo* and has been interpreted as lack of selection (e.g. Lescure 1964). The higher dry mass in arable and pasture may also reflect the higher percentage of hard bodied organisms (such as Coleoptera) in boluses from these habitats.

5.5.2 Sexual Dimorphism In The Diet Of *B. bufo*

The stomach contents of females had a significantly greater mass than those of males (Figure 5.3a). Females contained a significantly lower dry mass of Diptera and a significantly greater dry mass of Coleoptera, Diplopoda and Opilones than males. The differences in percentage abundance of dietary taxa between the sexes were small; only Diplopoda + Chilopoda had a higher percentage abundance in the diet of females than in males. Differences in dry mass of taxa were mainly due to the greater total dry mass in the stomach contents of females. Females were longer and had a significantly higher mass than males and there was a significant correlation between length of toad and

mass of stomach contents. This suggests that sexual differences in stomach contents are mainly a result of a sex-linked size dichotomy.

The sex-linked size dichotomy in some anurans has been linked to the cost of egg production to females and a concomitant delay in sexual maturation. The extra 1-3 years feeding, and predation based mortality can result in fewer, larger females compared with males within the breeding population (Jorgensen 1986). Gittins (1987) found no significant relationship between the size of food items in the diet and the size of adult *B. bufo* but he did not measure total dry mass of stomach contents. Pellantova (1978) and Gittins (1987) found the stomach contents of male and female *B. bufo* from a range of sites were similar. However they only considered percentage abundances and occurrences and not dry mass of stomach contents. Sexual dimorphism in prey selection in the anuran, *Adelotus brevis* was linked to sex related differences in habitat use and therefore availability of prey (Katsikaros and Shine 1997).

5.5.3. Prey Selection By *B. bufo*

Though comparisons between habitats and years were corrected for digestion using the indices, the percentages used in the electivity indices were not adjusted. Therefore all discussions of electivity must be judged with the caveat that soft bodied organisms such as Oligochaeta will digest more quickly than chitinous invertebrates such as Coleoptera. These soft bodied organisms will therefore be under-represented in the diet. The diel and nocturnal electivity indices of Coleoptera, Dermaptera, Hymenoptera and Diplopoda+Chilopoda were positive. The diel and nocturnal electivity indices for Acari and Collembola were negative(<-0.2).

These indices must be interpreted with caution as the prey availability measurements were subject to the errors inherent in invertebrate sampling using pitfall and suction traps (chapter 4). Pooling all data from the two different trap types and from different habitats should reduce these errors. *B. bufo* from outside the pitfall trapped area were used in 1995 and so prey availability may have differed for these *B. bufo*. The small

number of animals sampled in 1993 and 1994 may have resulted in sampling bias, again potentially affecting the electivity indices.

Despite the problems with sampling methods and the apparent plasticity of the diet of *B. bufo* from different habitats, the data demonstrated selection for fast moving prey. Lescure (1964) and Larsen (1984) suggested *B. bufo* preferred fast moving arthropods. The importance of mobility in the prey selection of anurans was also noted by Zimka (1966). He found that *Rana arvalis* had a distinct preference for predatory forms such as Carabidae, Formicidae, Aranae and Opilones. The chief factor in preference for predators was prey mobility. The direction of electivity (positive or negative) for most of taxa was consistent between years for both diel and nocturnal traps (Figures 5.5 and 5.6). The strongest positive electivity index was for Coleoptera. Selection of Coleoptera and Dermaptera by *B. bufo* above percentages available has been demonstrated by Cornish (1992). Most of the Coleoptera in the diet were Curculonidae and Carabidae (Section 5.4.2.). Carabidae are active ground moving predators (Greenslade 1964, Baars 1979) and are therefore more likely to be encountered by a toad that has adopted a sit-and-wait strategy than are more sessile invertebrates. Curculonidae are herbivorous but are active and cursorial (Unwin 1984). Carabidae and Curculonidae have been shown to be important constituents of *B. bufo*' diets in previous studies (e.g. Pellantova 1978, Gittins 1987, Larsen 1984).

Coccinelidae the third most abundant Coleopteran in diet of *B. bufo* are not cursorial, and are usually found on low lying vegetation. The predation of Coccinelidae, Tipulidae and Dermaptera by *B. bufo* indicates that *B. bufo* were not restricted to cursorial feeding but were able to climb on vegetation (e.g. Denton 1991).

In the current study there was a preference for larger over smaller taxa. Collembola and Acari were well represented in all habitats but were infrequent and contributed no significant mass to the diet. This may be because Collembola were soft bodied and would be difficult to identify when digested. Acari, however, digest very slowly due to their chitinous bodies and would therefore be better represented in the stomach contents. Collembola and Acari may be difficult for larger adult *B. bufo* to detect. The

number of receptory elements per square unit of retina is reduced as eye size in the toad increases with a concomitant reduction in the accuracy of vision (Grobstein *et al* 1983). Acari and Collembola are eaten by juvenile *B. bufo* (Cornish 1992) and the differences in species and size of prey eaten by juvenile and adult toad may reduce the level of intraspecific competition.

Behaviour of invertebrates with respect to microclimate may have influenced the electivity indices. The correlation of total invertebrate numbers with minimum temperature in chapter 4, demonstrated the importance of microclimate in invertebrate activity. Isopoda had positive diel indices in all 3 years and were the 2nd most abundant taxon in the diets of *B. bufo* from woodland and arable habitats (Figure 5.5). Isopoda are very susceptible to water loss and are nocturnal (Hopkin 1991). Isopoda are active on warm wet nights; conditions which correspond with peaks in toad activity (e.g. Grist 1994). However Isopoda have circum-neutral nocturnal electivity indices therefore this taxa is abundant in the diet probably because Isopoda activity patterns coincide with those of the toad (Figure 5.6). Carabidae are also sensitive to soil structure and to microclimate, Carabidae activity will be highest on warmer nights (Walsh 1990) and Coleoptera have high positive diel and nocturnal indices. This suggests that Coleoptera are selected even above their high nocturnal percentage abundances. The interplay between microclimate, prey availability, foraging mode, habitat and prey selection are discussed in chapter 8.

5.6 CONCLUSIONS

- Female *B. bufo* were longer and had higher masses than males but size (mass and length) of *B. bufo* did not vary significantly between capture habitats.

- There was a significant positive correlation between *B. bufo* length and total dry mass of stomach contents.

- The percentage abundance of taxa in the diet of male and female *B. bufo* differed significantly.

- The diet of *B. bufo* was dominated, in terms of both percentage abundance and dry mass, by Coleoptera. Coleoptera, Opilones + Aranae, Hemiptera, Diplopoda + Chilopoda, Diptera and Hymenoptera each had abundances of greater than 10%.

- The diet of *B. bufo* differed between habitats reflecting differences in the prey captures by pitfall and suction traps in these habitats. There were higher dry masses of Coleoptera in the stomachs of *B. bufo* from arable+pasture than those from woodland+rough grassland habitats.

- Coleoptera had the most positive diel and nocturnal electivity indices in all years. Collembola and Acari had strong negative diel and nocturnal indices. This suggests that *B. bufo* select larger prey over small invertebrates such as Acari.

6: SURVIVAL IN DIFFERENT HABITATS

Habitat may influence survival directly by providing microclimates which ameliorate climate extremes or indirectly via an affect on prey (chapters 4 and 5) and predation. Numbers of predators may vary between habitats and susceptibility to predation may also differ with habitat. The influence of microclimate and predation on survival *of B. bufo* are described below in sections A and B respectively.

Section (A) describes the microclimate in four different habitats within the radio-tracking area (described in chapter 3) and relates this to the movement of radio-tracked *B. bufo.* Habitat specific experimental enclosures were used to compare survival and desiccation rates in different habitats. To evaluate predation risk the same enclosures were used to compare the ease of sighting *B. bufo* in different habitats (described in Section B). Mortality data recorded at 26 sites during the breeding season and mortality of radio-tracked *B. bufo* is included in section B. The interaction between these microclimatic and predation variables will be discussed in chapter 8.

(A) MICROCLIMATE AND SURVIVAL

6.1 INTRODUCTION

The abiotic and biotic factors within an ecosystem interact: the geographical range of a species may be limited by climate but on a local scale vegetation can modify the ambient temperature and humidity producing habitat-specific microclimates. Amphibians are particularly sensitive to the microclimate as they are ectothermic. Most amphibians also have a thin water-permeable epidermis and when placed in a desiccation chamber will demonstrate evaporative water loss at rates similar to that of standing water (Kirk and Hogben 1946). Water economy is therefore fundamental to amphibian biology.

Thorson and Svihla (1943) determined the lethal limits of water-loss for ten species of amphibians from ten different habitats; from xeric adapted *Scaphiopus* species, to the aquatic *R. grylio.* Terrestrial species were able to survive higher percentage water loss than more aquatic species and the ability to survive desiccation stress increased with

aridity of habitat. For example *Scaphiopus* were able to survive up to 60% loss of body water compared with 40% in the semi-aquatic *R. grylio*. Thorson (1955) found that rates of water loss decreased with the mean size of the species. The ability to survive high percentage of water loss decreased with initial mass of fully hydrated animals because the percentage of body water decreased with increased size. For example small *R. pipiens* (5g -14g) survived an average of 45% loss of body water compared with the 32% loss of body water survived by the large *R. pipiens* (60 - 74 g) (Thorson 1955).

Terrestrial amphibians typically rehydrate by cutaneous water absorption through a specialised area of the ventral surface that is bought into contact with moist substrates during a process termed "cutaneous drinking"(e.g. Jorgensen 1991a, 1994a, 1994b). Baldwin (1974) named this region of highly vascularised skin, in contact with the ground, the pelvic patch. Rapid rehydration depends on rich vascularisation in the pelvic patch which serves to maintain a high osmotic gradient across the epithelium (Roth 1973). In bufonids and other anurans sculpturing of the skin carries water by capillary forces from the ventral surface over the rest of the body (Christensen 1974, Cree 1989) and increases the area of skin in contact with the substrate (Hillyard 1988). The ability to rehydrate via cutaneous drinking varies between species and with substrate moisture content. Xeric adapted species such as the north-American spadefoot toad (*Scaphiopus*) can absorb water at soil water tensions of up to 15 atm (Ruibal *et al* 1969). Mesic-adapted *B. americanus* lose water to the soil at soil water tensions above 1.5 atm (Walker and Whitford 1970). Water moves passively across amphibian skin and the direction of water flow between the substrate and the body is determined by the water potential across the skin. Amphibians are able to rehydrate at low water potentials because of the coupling of kidney, bladder and skin functions.

When amphibians are in a water-acclimated state, water is absorbed through the skin passively at a relatively low rate, excreted by the kidneys and stored in the bladder as a highly dilute urine before voiding (Jorgensen and Rosenkilde 1956, Jorgensen e*t al* 1969, Jorgensen 1994a, 1994b, 1997). Water may be resorbed from the bladder and thus recirculated. The low rates of in-vitro water resorption (12mg $cm^{-2}h^{-1}$) suggest

that this process is of minor importance in a water acclimated animal (Matty and Green 1962).

Outside the breeding season terrestrial amphibians may have little contact with open water. Water resorption from the bladder becomes increasingly important in more arid conditions (Jorgensen 1997): When *R. temporaria* was in contact with wet soil, diuresis equalled water influx and so resorption of water from the bladder was insignificant (about 6% of the diuresis) (Sinsch 1987b). When the water potential of soil was -5.5 bar, water from the bladder was resorbed and water influx was 1/10th of the influx of water into frogs on wet soil (Sinsch 1987b). Urine is not normally voided on land and urine flow of *B. bufo* starts to decline shortly after transition from water to a dry or isosmotic environment (Jorgensen 1994a) Reduction of permeability in the bladder wall in response to drying of microclimates allows amphibians, including *B. bufo,* to remain hydrated on land (Jorgensen 1991a, 1991b 1994a).

Despite cutaneous drinking and coupling of kidney, bladder and skin functions, amphibians are prone to dehydration via evaporative moisture loss especially in their summer home ranges. They usually display behaviour that minimises water loss. Amphibians are typically nocturnal or crepuscular animals that forage when air temperature is low and humidity correspondingly high. Thorson and Svihla (1943) showed that, when exposed to a desiccation chamber, behaviour of ten species of anurans from many different habitats was fairly constant; an attempt to escape followed by adoption of a hunched minimum surface area position. This evaporation-avoiding posture is adopted by *B. bufo* and is interrupted intermittently by water seeking or escape behaviour (Jorgensen 1991a).

In extreme or arid environments physiological adaptations to prevent desiccation have developed. Arboreal tree frogs such as the South African tree frogs (*Chiromantis* species) and reed frogs (*Hyperolius* species) have less permeable skin than other amphibian species (e.g. Poynton 1964). *Litoria caerulea* uses lipid skin secretion as a barrier to desiccation over dorsal surfaces (Christian *et al* 1988). In extremely arid environments some animals may become dormant for the dry season. Aestivational

cocoons made of single or multiple layers of shed strata cornea reduce losses of water into the soil (Lee and Mercer 1967, Van Beurden 1984).

6.2 APPROACH AND AIMS

A method for assessing the effects of habitat microclimate on survival and mass change was tested in 1993. This method was then applied in two separate experiments in May and June of 1995. The relationship between habitat choice and microclimate was assessed by relating the behaviour of radio-tracked *B. bufo* (described in chapter 3) to the microclimate within the radio-tracking enclosure.

6.3 THE PRELIMINARY INVESTIGATION

6.3.1 Introduction

Since the body mass and water content of *B. bufo* are so closely related, short term mass changes (<1 week) reflect the change in the ratio of hydration state to feeding. Moisture loss was assumed to be correlated with short term mass loss of *B. bufo*. Pasture, rough grassland and woodland habitats were chosen to coincide with three of the four habitats in the radio-tracking enclosure. The preliminary investigation was set up to assess the experimental design. The primary aim was to determine if *B. bufo* without radio-transmitters would be locatable in outdoor enclosures. Secondly to assess if enclosures were escape-proof, as loss of animals from the enclosure would invalidate the experiment.

6.3.2 Methods

Three 2m x 2m netlon enclosures were constructed at the edge of an old hay meadow, in Coleorton (SK 396168). The three enclosures were given the following habitat titles: short grassland, rough grassland and woodland. The hay meadow was cut to 0.5m to form the rough grassland. The meadow grasses were closely cropped from 50cm to a height of less than 2cm and the clippings removed to simulate pasture. The woodland enclosures were built beneath pine and oak trees (*Pinus sylvestris and Quercus petraea*). Beneath the trees in the woodland enclosure were mainly bramble, ivy and senescent raspberry bushes. All enclosures were within 50m to ensure that

abiotic variables not related to habitat (such as slope, sun aspect and rainfall) were nearly identical in each enclosure.

Seven *B. bufo*, marked individually using a "Panjet", were placed into each of the three enclosures. The habitats were searched daily for a total of seven days and mass of all retrieved *B. bufo* was measured to an accuracy of 0.1g (+/-0.01g) using a battery powered field balance. The enclosure design is shown in Figure 6.1a.

6.3.3 Statistical Analysis

The number of *B. bufo* retrieved in different habitats were compared using Chi-square test. The mass of *B. bufo* in different habitat enclosures were compared using a repeated measures ANOVA.

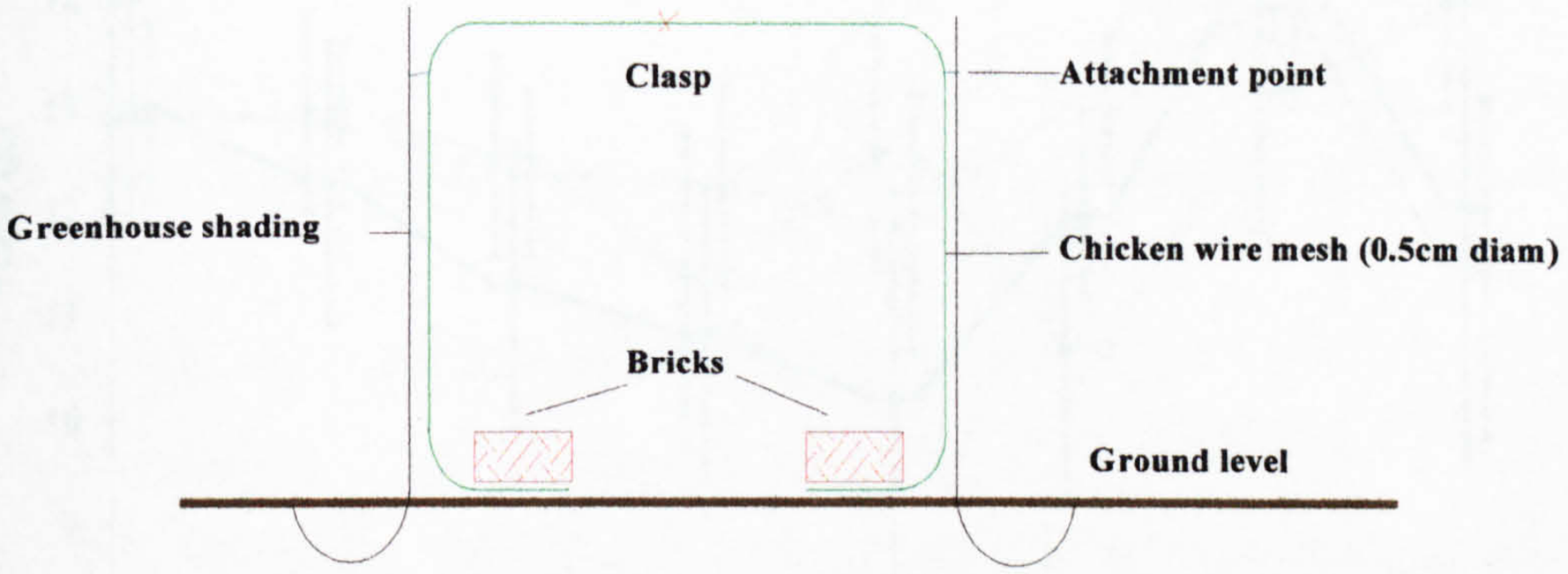
6.3.4. Results

No corpses were found in the rough grassland or woodland enclosures. By the end of the experiment two *B. bufo* were not retrieved from the rough grassland and woodland enclosures. At the end of the experiment two corpses of *B. bufo* were found in the short grassland enclosure and two *B. bufo* were not retrieved from this enclosure. There was not a significant difference in retrieval between habitat types ($\chi^2_{2,18}$=0.61, P>0.05). The mean masses of *B. bufo* in each enclosure are shown in Figure 6.2. Mean mass of *B. bufo* did not differ significantly with enclosure habitat ($F_{2,18}$=0.26, P>0.05) or with time ($F_{6,42}$=0.68, P>0.05). Figure 6.3 shows the number of *B. bufo* retrieved per habitat per day. The recovery rates on day 1 were 90% but had declined to 21% by day 7.

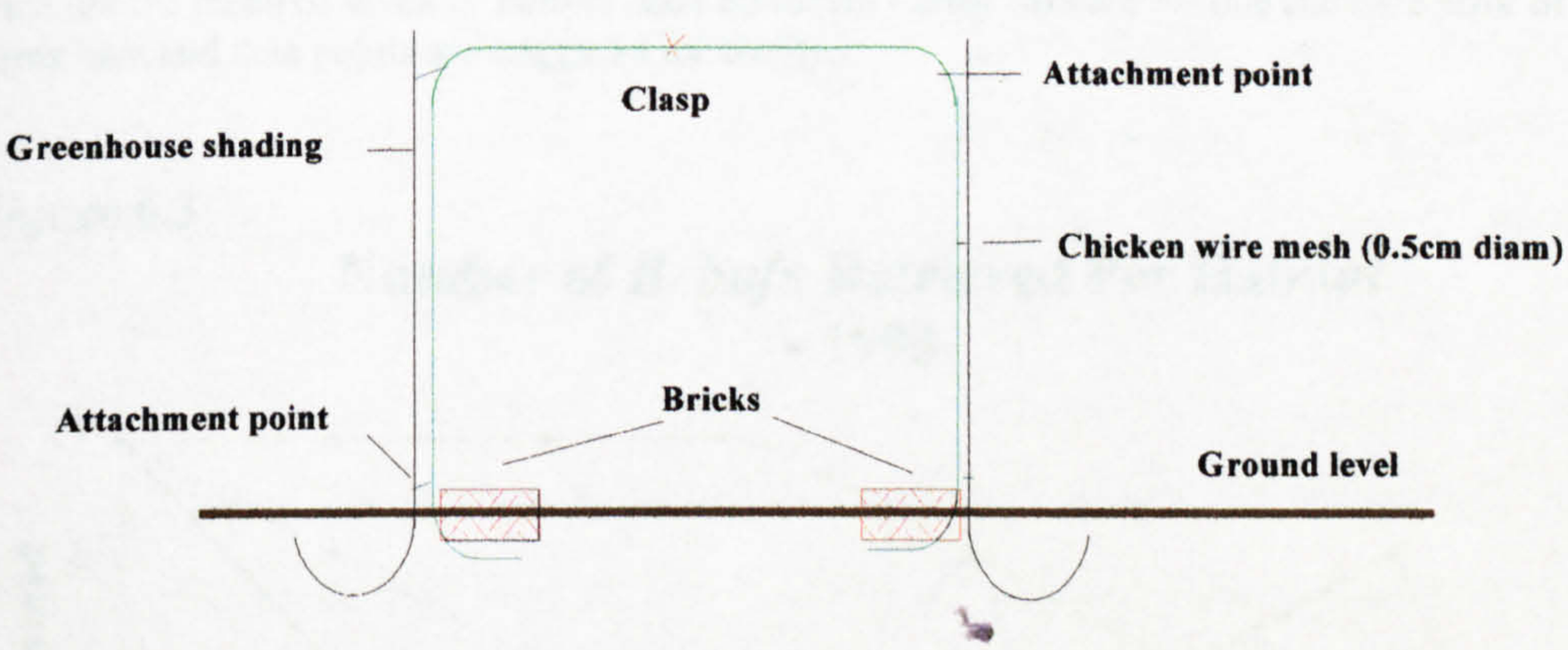
Figure 6.1

Design of Experimental Enclosures In 1993 And 1995

Enclosure Design - 1993



Enclosure Design - 1995



Enclosure Design In 1993

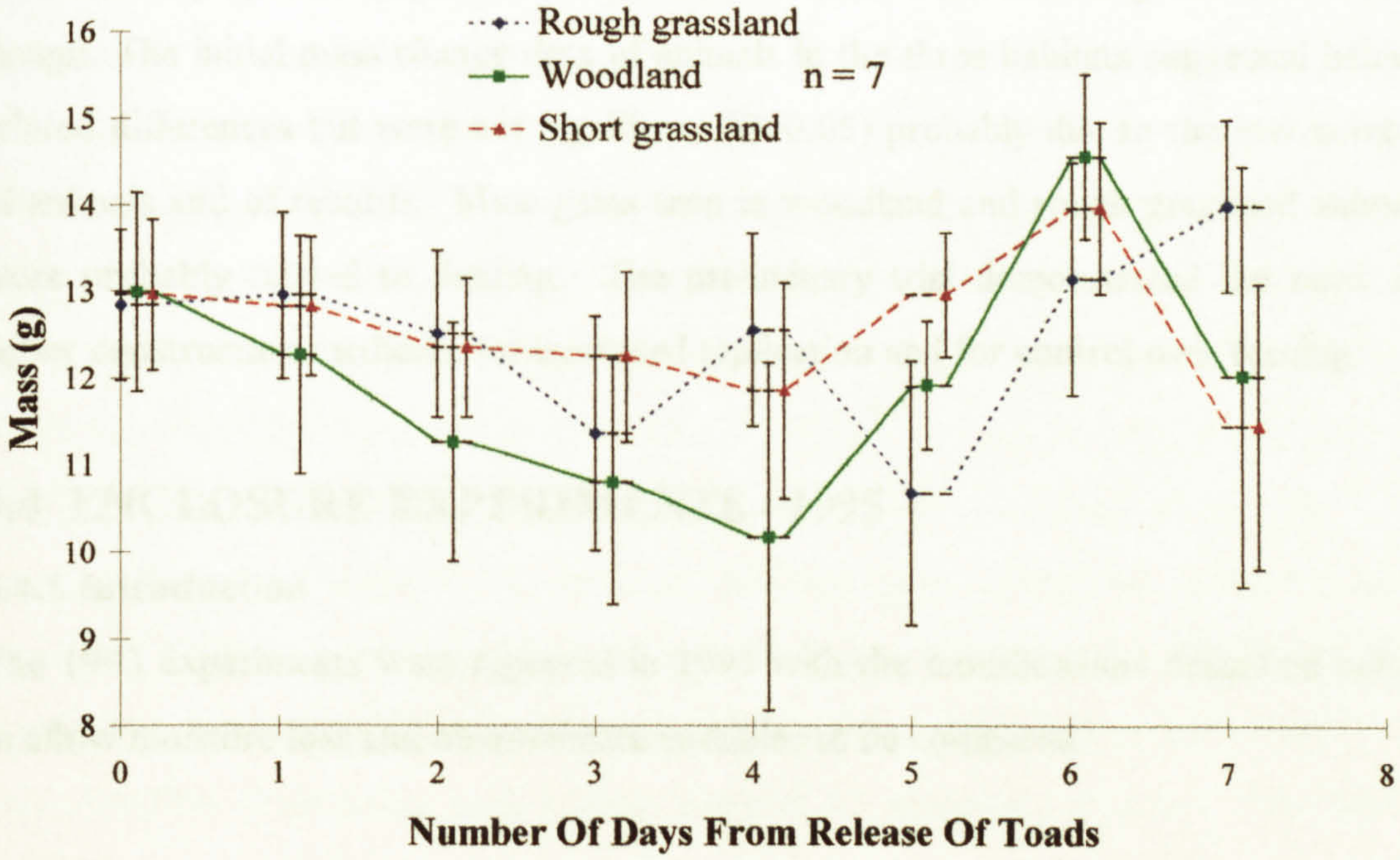
The enclosures were 2m x 2m at the base and 80cm high. The chicken wire outer fence was buried at the base. The inner fence and roof were made of greenhouse shading. The roof, inner and outer fences were all stitched together at the top of the outer fence. The inner was held down by bricks at the base of the enclosure.

Enclosure Design In 1995

The basic design followed that of 1993 with the following modifications: In 1995 the inner and outer fences were stitched at the base. The greenhouse shading and the bricks at the base of the inner fence were buried to prevent escape of *B. bufo*.

Figure 6.2

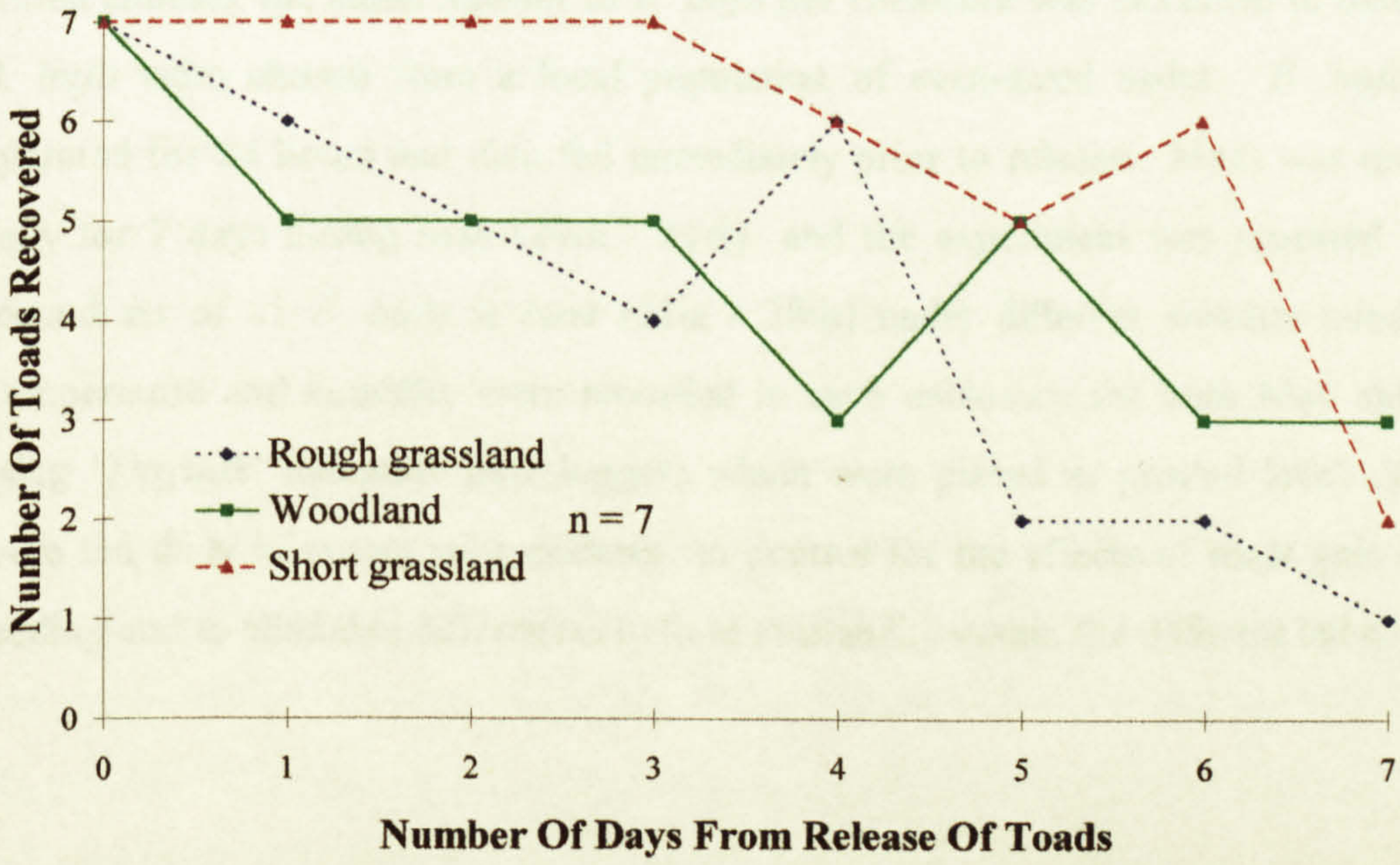
Mean Mass Of *B. bufo* In Different Habitats - 1993



Data are the mean of seven *B. bufo* in each enclosure - error bars are +/- one standard error of the mean. Error bars and data points are staggered for clarity.

Figure 6.3

Number of *B. bufo* Retrieved Per Habitat - 1993



Seven *B. bufo* were released into each of the 3 enclosures on day 0.

6.3.5 Discussion

The animals not retrieved at the end of the experiment can probably be ascribed to animals escaping from gaps at the base of the enclosure necessitating a new enclosure design. The initial mass change data of animals in the three habitats suggested habitat related differences but were not significant ($P>0.05$) probably due to the low number of animals and of records. Mass gains seen in woodland and rough grassland animals were probably related to feeding. The preliminary trial demonstrated the need for better construction methods, for increased replication and for control over feeding.

6.4 ENCLOSURE EXPERIMENTS - 1995

6.4.1 Introduction

The 1993 experiments were repeated in 1995 with the modifications described below to allow moisture loss and microclimate variables to be compared.

6.4.2 Methods

The enclosures were redesigned by sewing the edges and roof of the netlon together and by burying the base with a combination of bricks and soil. The modifications to the enclosure design are shown in Figure 6.1a and b. Three replicate enclosures of each habitat type were used. To allow for mortality and the difficulty of recovering buried animals, the initial number of *B. bufo* per enclosure was increased to nine. The *B. bufo* were chosen from a local population of even-sized males. *B. bufo* were hydrated for 24 hours and then fed immediately prior to release. Mass was recorded daily for 7 days during May (24th - 31st) and the experiment was repeated with a second set of 81 *B. bufo* in June (21st - 28th) under different weather conditions. Temperature and humidity were recorded in each enclosure for both May and June using '*Tinytalk*' miniature data loggers which were placed at ground level. Animals were fed daily in excess with crickets to control for the effects of mass gain due to feeding and to eliminate differences in food availability within the different habitats.

6.4.3 Analysis

The mass of retrieved *B. bufo* from the experiments in May and June were used in two two-way repeated measures ANCOVA with enclosure replicate and habitat type as the independent variables with initial mass of *B. bufo* as the covariate. Initial mass of *B. bufo* was regressed against final percentage mass loss. Daily mass change was calculated as the mass on day n+1 - mass on day n for each day from day 0 to day 6. Mean, minimum and maximum daily temperatures and humidity in each enclosure were regressed against daily mass change using stepwise multiple linear regression followed by a modified Bonferroni correction (Simes 1986) to reduce type I errors from multpiple tests. The Simes Bonferroni correction was discussed in section 3.4.2. Temperatures and humidity in different habitats were compared using repeated measures ANOVA with enclosure replicate and habitat type as independent variables. Percentage survival in different habitats was compared using a non parametric two-way ANOVA (Friedman) with habitat blocked by enclosure replicate. The abiotic variables in the two experiments were compared using paired T-tests for mean, minimum and maximum humidity and temperature followed by Bonferroni correcting for multiple tests. Post-hoc multiple means (Tukey) tests were applied where necessary.

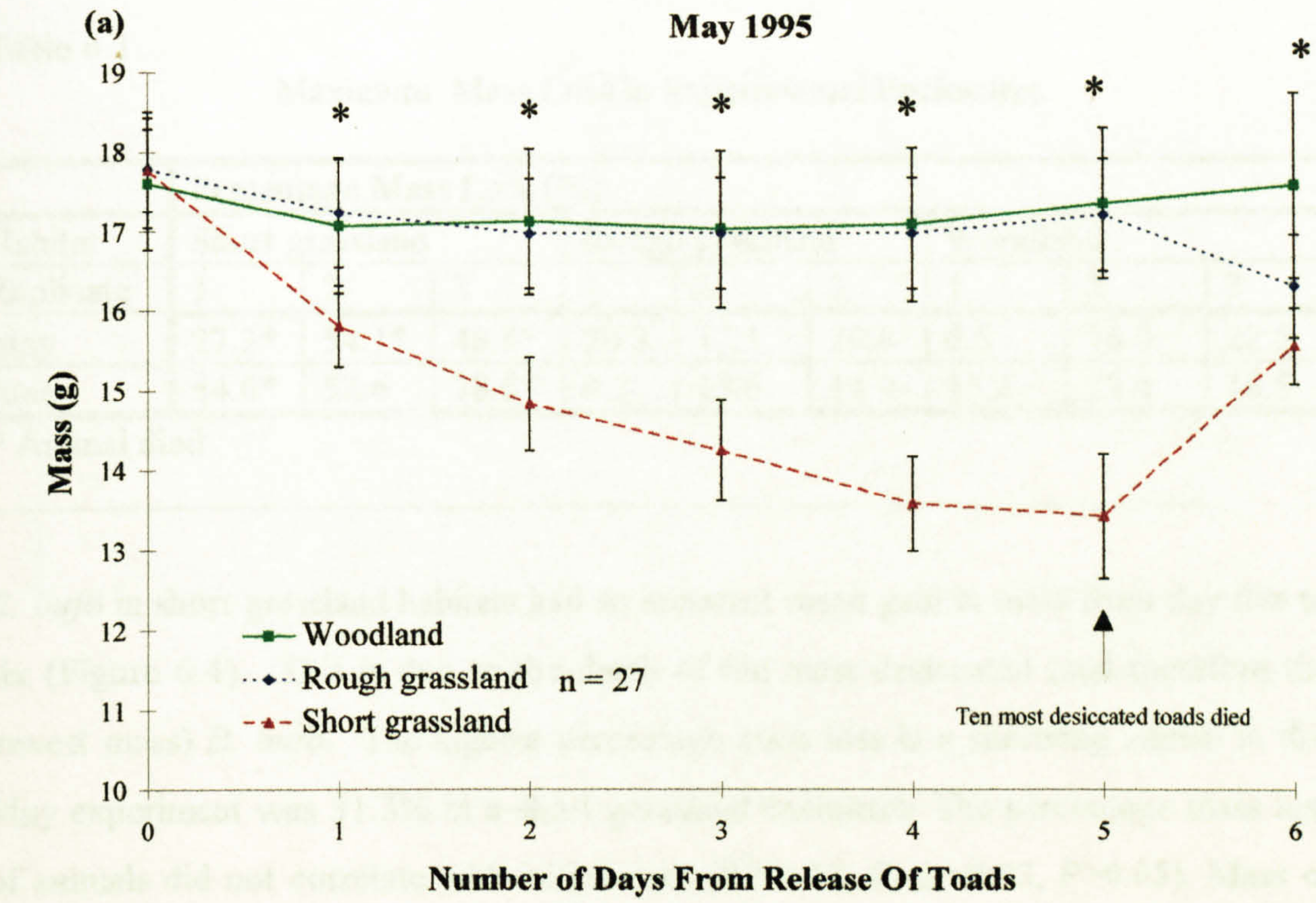
6.4.4 Results

(i) Enclosure Experiment 1 May 1995

(ia) Mass And Survival

Significantly fewer *B. bufo* survived in short grassland than the other two habitats ($S_{2\ adj\ for\ ties}$=6.00, P<0.05). All nine animals survived in each of the three woodland and three rough grassland enclosures, ten animals died due to desiccation stress in the short grassland. The mean mass of *B. bufo* per habitat is shown in Figure 6.4 and the maximum percentage mass loss of *B. bufo* is given in Table 6.1. Mass of *B. bufo* did not differ between replicate enclosures ($F_{2,52}$=0.75, P>0.05). *B. bufo* in short grassland lost significantly more mass than those in woodland and rough grassland enclosures ($F_{2,52}$=3.59, P<0.005). Mass varied daily ($F_{4,208}$=8.72, P<0.001) mainly due to the reducing mass of *B. bufo* in short grassland ($F_{8,208}$=4.30, P<0.001).

Figure 6.4



Data are the mean values for three replicate enclosures each with nine animals per enclosure released on day 0. * are significant between-habitat differences in mean mass of *B. bufo*. Error bars are +/- one standard error of the mean.

Table 6.1

Maximum Mass Loss in Experimental Enclosures

	Percentage Mass Loss (%)								
Habitat	Short grassland			Rough grassland			Woodland		
Replicate	1	2	3	1	2	3	1	2	3
May	27.3*	54.1*	48.5*	20.3	17.1	20.8	6.5	16.0	22.5
June	44.6*	58.6	78.6*	9.3	13.6	14.9	15.2	29.4	16.5

* Animal died

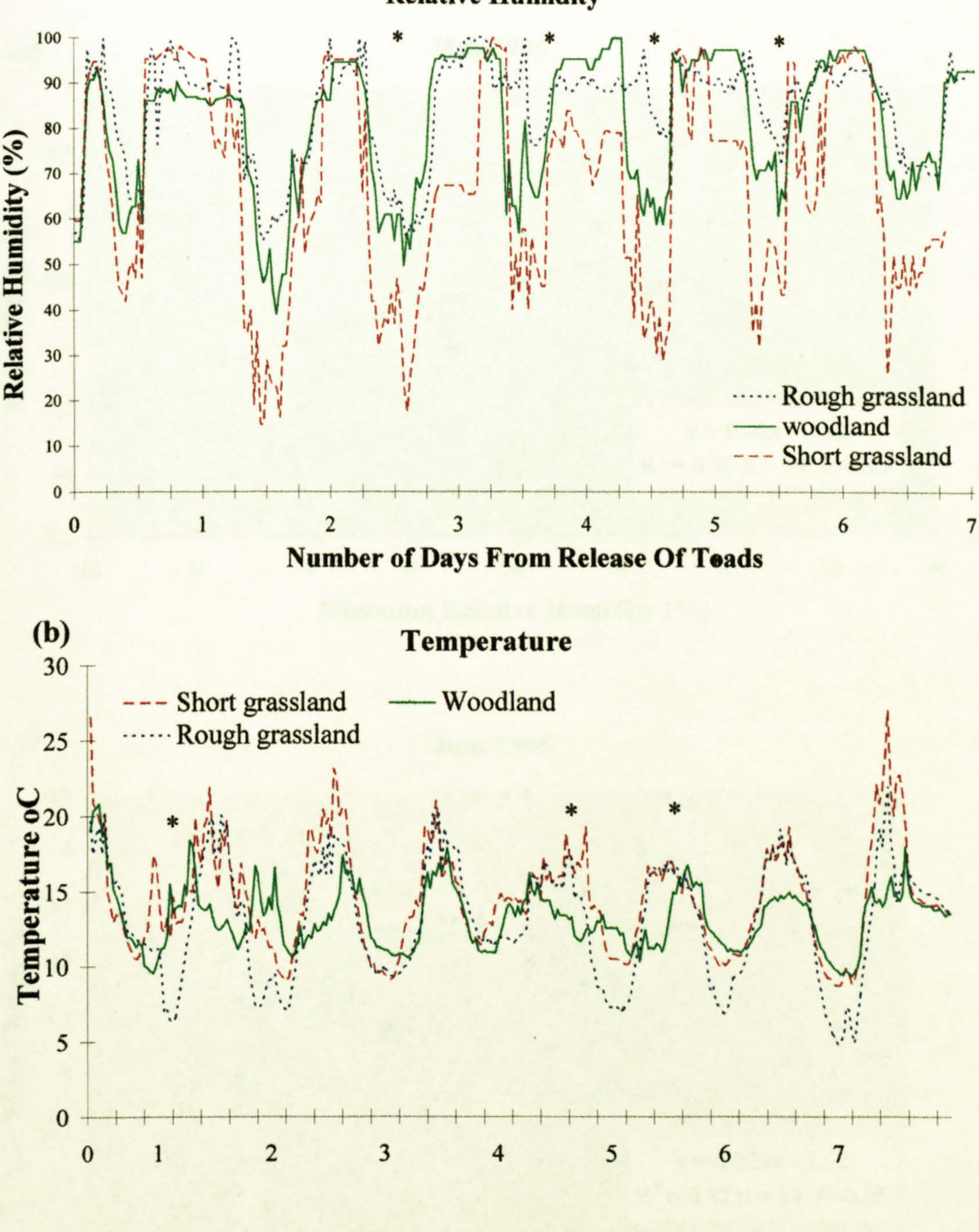
B. bufo in short grassland habitats had an apparent mean gain in mass from day five to six (Figure 6.4). This is due to the death of the most desiccated (and therefore the lowest mass) *B. bufo*. The highest percentage mass loss in a surviving animal in this May experiment was 31.3% in a short grassland enclosure. The percentage mass loss of animals did not correlate with initial mass (R^2=0.12, $F_{1,143}$=0.93, P>0.05). Mass of *B. bufo* from 1993 experiments and both 1995 experiments are given in Appendix D tables 1,2 and 3.

(ib) Abiotic Variables

Temperature and humidity varied with habitat ($F_{2,351}$=2.91, P<0.06; $F_{2,351}$=125.89,P<0.001 respectively). Both temperature and humidity varied daily ($F_{7,1755}$=504.04,P<0.001; $F_{7,1755}$=87.98, P<0.001 respectively). Daily fluctuations in temperature and humidity were influenced by habitat ($F_{14,1755}$=3.47,P<0.001; $F_{14,1755}$=13.05, P<0.001 respectively). Mean humidity and temperature are shown in Figures 6.5 a and b. On all days woodland and rough grassland were more humid than short grassland and on days three, five, six and seven woodland was more humid than rough grassland. Short grassland had higher mean and maximum temperature than woodland on all days. Rough grassland had higher temperatures than woodland on days five and six. Minimum humidity correlated significantly with mass change of *B. bufo* (R^2=0.50, $F_{1,54}$=10.27, P<0.01). The regression of minimum humidity and mass change is shown in Figure 6.6a.

Figure 6.5

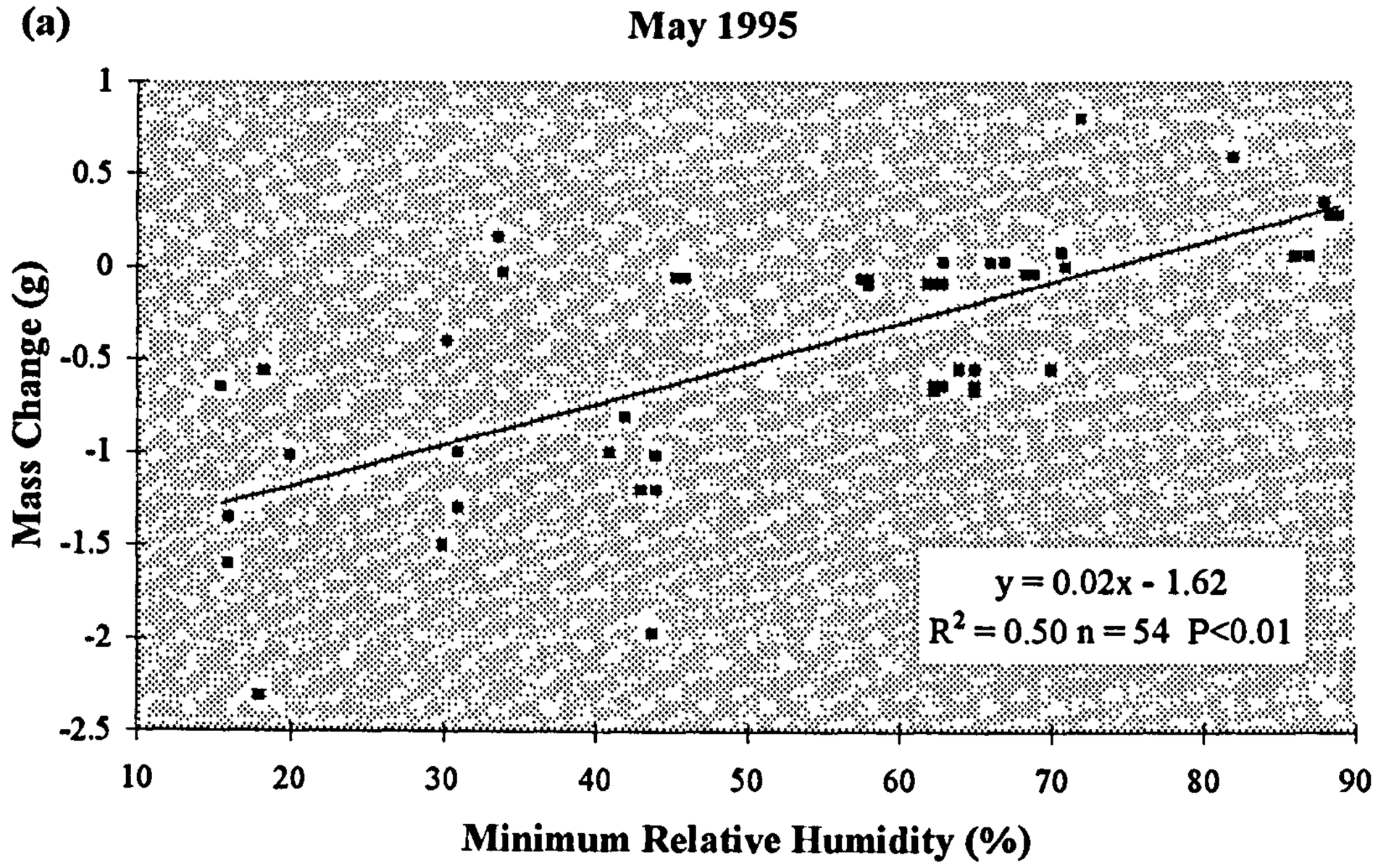
Microclimate At Ground Level In Habitat Enclosures -May 1995

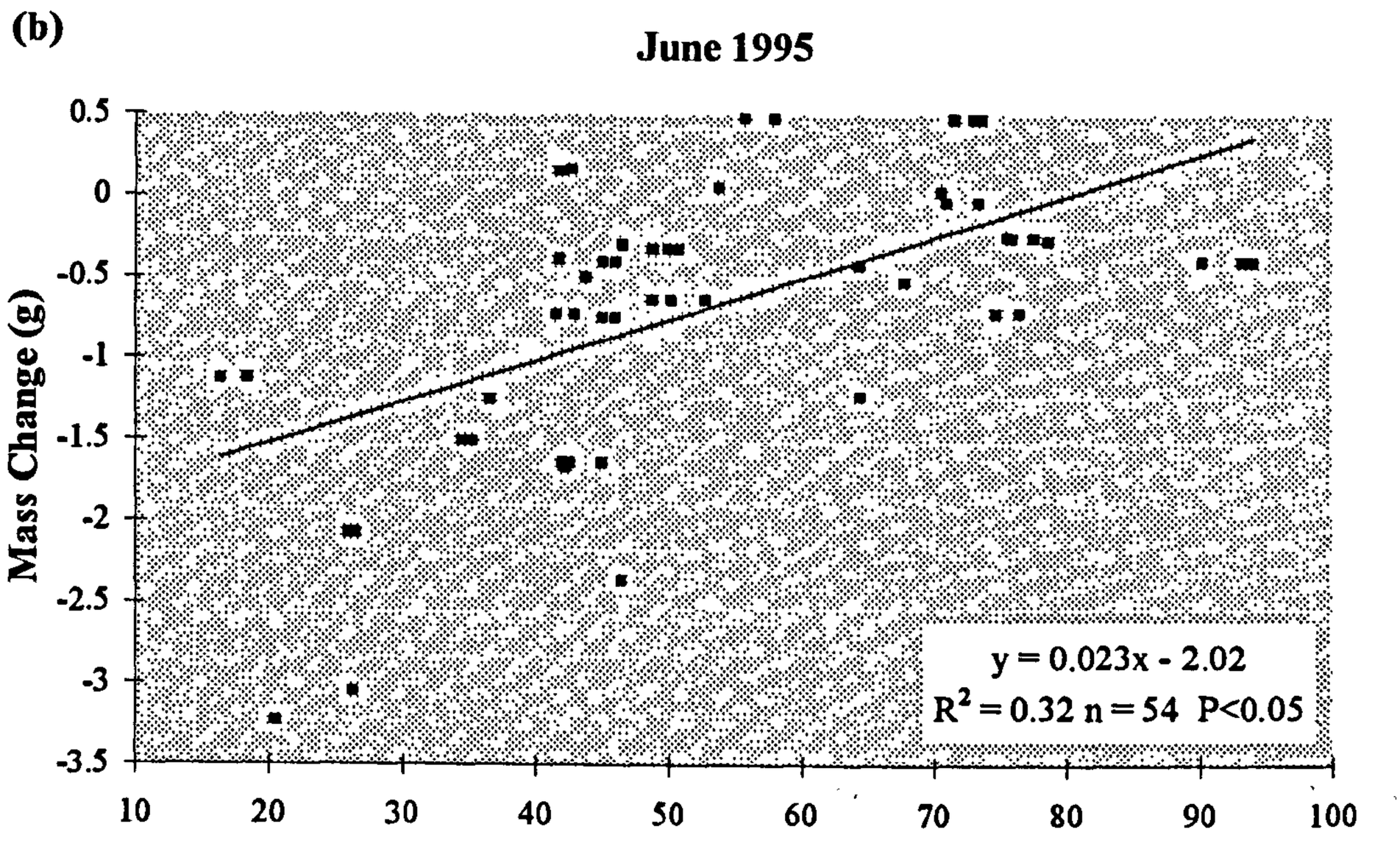


In each habitat mean values from three replicate enclosures are plotted. * are days when temperature/humidity in woodland differed significantly from those in rough grassland enclosures ($P<0.05$). Humidity and temperatures in short grassland differed significantly from those in woodland on all 7 days ($P<0.05$). Temperature and humidity were recorded using "*Tinytalk*" data loggers.

Figure 6.6

Minimum Relative Humidity And Mass Change Of Common *B. bufo* During One Week Of Observations





Mass change of each toad = mass of toad on day n+1 - mass of toad on day n. Mean mass change of *B. bufo* within an enclosure was paired with humidity on day n. Humidity was recorded using "*Tinytalk*" data loggers. n-1 days = 6. 6 x 9 enclosures = 54.

There were no correlations between any of the other abiotic variables and daily mass change ($P_{(all)}$ >0.05). Both temperature and humidity showed large diurnal variation. In short grassland diurnal temperature variation was as high as 25°C (May 31st).

(ii) Enclosure Experiment 2 - June 1995

(iia) Toad Mass And Survival

Significantly more *B. bufo* died in short grassland than the other two habitat types ($S_{2\ adj\ for\ ties}$=6.00, P<0.05). All nine animals survived in each of the three woodland and three rough grassland enclosures. 11 animals died of desiccation stress in the short grassland. The mean mass of *B. bufo* per habitat is shown in Figure 6.4b and the maximum percentage mass loss of *B. bufo* is given in Table 6.1. *B. bufo* in short grassland enclosures lost more mass than *B. bufo* in the other habitat enclosures ($F_{2,46}$=2.47, P<0.01). Mass varied daily ($F_{6,230}$=6.71, P<0.001) mainly due to the significant decrease in mass of *B. bufo* in short grassland ($F_{12,230}$= 6.71, P<0.001). Mass of *B. bufo* in replicate enclosures did not differ significantly ($F_{2,46}$=0.01, P>0.05). The percentage mass loss of animals did not correlate with initial mass (R^2=0.01, $F_{1,134}$=0.01, P>0.05). The highest percentage mass loss in a surviving animal in this June experiment was 58.6% in a short grassland enclosure.

(iib) Abiotic Variables

Temperature and humidity each varied with habitat ($F_{2,351}$=2.81, P<0.1; $F_{2,351}$=2.49, P<0.01 respectively). Both temperature and humidity varied significantly between days ($F_{6,2106}$=82.04, P<0.001; $F_{6,2106}$=373.42, P<0.001). Daily fluctuations in temperature and humidity were influenced by habitat ($F_{12,2106}$=6.48, P<0.001; $F_{12,2106}$=13.28, P<0.001). Mean humidity and temperature of the 3 enclosures are shown in Figure 6.7 a, b. On six days short grassland had higher mean and maximum temperatures than woodland and on one day short grassland had significantly higher temperatures than rough grassland. On two days rough grassland had significantly higher temperatures than woodland. On two days woodland was more humid than short grassland and on one day rough grassland was more humid than short grassland. There was a significant correlation between minimum humidity and mass change of *B. bufo* (R^2=0.32, $F_{1,54}$=7.96, P<0.05). The regression between minimum humidity and mass change is shown in Figure 6.6b.

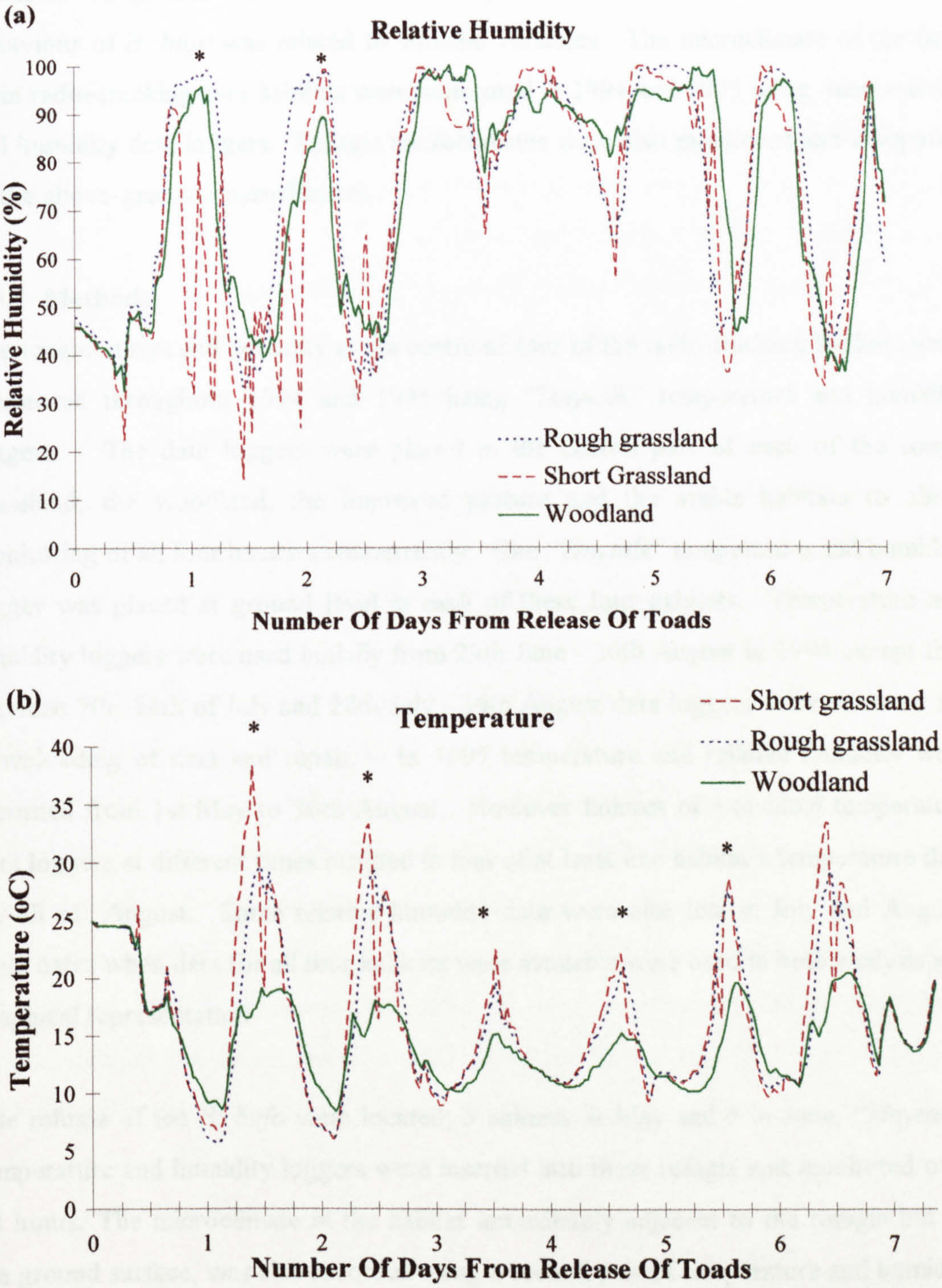
Diurnal variation was high in both temperature and humidity. The maximum daily variation in temperature was 35°C in the short grassland (June 22nd). The regression of minimum humidity and mass change is shown in Figure 6.6b. There were no correlations between any of the other abiotic variables and daily mass change ($P_{(all)}$ >0.05).

(iic) Comparison Of Observations In May And June

Mass changes of *B. bufo* were higher in June than in May but the difference was not significant ($T_{1,17}$=2.05,P=0.56). Figure 6.4 b shows the decrease in toad mass in all habitats in June. In May *B. bufo* only lost mass in short grassland habitats (Figure 6.4b). Mean, minimum and maximum humidity in each enclosure did not differ significantly between May and June ($T_{1,13}$ = 0.70, 0.23, -1.13, $P_{(all)}$ >0.05). Maximum, minimum and mean temperatures were all significantly higher in June than in May ($T_{1,13}$= -3.16, -3.58, -2.58, respectively $P_{(all)}$ <0.05).

Figure 6.7

Microclimate At Ground Level In Habitat Enclosures - June 1995



In each habitat mean values from three replicate enclosures are plotted. * are days when temperature/humidity differed significantly between habitats($P<0.05$). Temperature and humidity were recorded using "*Tinytalk*" data loggers.

6.5 MICROCLIMATE AND HABITAT PREFERENCE

6.5 1 Approach And Aims

These investigations determine whether microclimate varied with habitat and if behaviour of *B. bufo* was related to climatic variables. The microclimate of the four main radio-tracking area habitats were monitored in 1994 and 1995 using temperature and humidity data loggers. Refugia microclimates were also monitored and compared to the above-ground microclimates.

6.5.2 Methods

The temperatures and humidity at the centre of four of the radio-tracking habitats were monitored throughout 1994 and 1995 using *"Tinytalk"* temperature and humidity loggers. The data loggers were placed in the central part of each of the rough grassland, the woodland, the improved pasture and the arable habitats to allow monitoring of all four habitats concurrently. One "*Tinytalk*" temperature and humidity logger was placed at ground level in each of these four habitats. Temperature and humidity loggers were used initially from 29th June - 30th August in 1994 except that between 7th -14th of July and 28th July - 14th August data loggers were removed for downloading of data and repair. In 1995 temperature and relative humidity were recorded from 1st May to 30th August. However failures of individual temperature data loggers at different times resulted in loss of at least one habitat's temperature data for all of August. Some relative humidity data were also lost in July and August. Only dates when data for all four habitats were available were used in both analysis and graphical representation.

The refugia of ten *B. bufo* were located; 5 animals in May and 5 in June. *"Tinytalk"* temperature and humidity loggers were inserted into these refugia and monitored over 24 hours. The microclimate in the habitat immediately adjacent to the refugia but on the ground surface, was also recorded using a second pair of temperature and humidity loggers. The microclimates of these "surface" habitats were compared to the microclimates in refugia. Due to data logger failure measurements from only six refugia were suitable for analysis. Data logger numbers were limited so microclimate recording was done sequentially for each refugia. A comparison between the

microclimate of male and female refugia habitats was therefore not possible. The surface microclimate was recorded in parallel with the recording of each refugium microclimate to allow comparison of surface and refugia microclimates.

6.5.3 Analysis

The mean, minimum and maximum daily temperatures were entered as the dependent variables using 2 way MANOVA with habitat and year (94/95) as the independent variables. A second MANOVA used the same independent variables but mean, minimum and maximum humidity were the dependent variables. Data from "*Tinytalks*" in refugia were compared with data from equivalent surface habitats by pairwise T-tests for temperature and humidity in 1994. Post hoc multiple means tests were applied where necessary. To assess if microclimate variables influenced behaviour of radio-tracked *B. bufo* a new variable was formed called "movement". Radio-tracking data from chapter 3 was used to identify periods when one or more *B. bufo* moved out of refugia. Each tracking period and the preceding diurnal period (since radio-tracking was mainly nocturnal) were termed a "movement day". Movement days were classified into two types: movement days on which one or more *B. bufo* moved into arable or pasture were type B days. Movement days when *B. bufo* movement was restricted within rough grassland and/or woodland and did not enter either arable or pasture were type A days. Woodland and rough grassland were paired as they were the most preferred habitats by radio-tracked *B. bufo* (Chapter 3). Arable and pasture habitats were paired as they were the least preferred habitats by radio-tracked *B. bufo* (Chapter 3). A MANOVA was performed using mean minimum and maximum daily temperatures as dependent variables and "movement" (A or B) as the factor. This was repeated for mean minimum and maximum humidity.

6.5.4 Results

(i) Relative Humidity

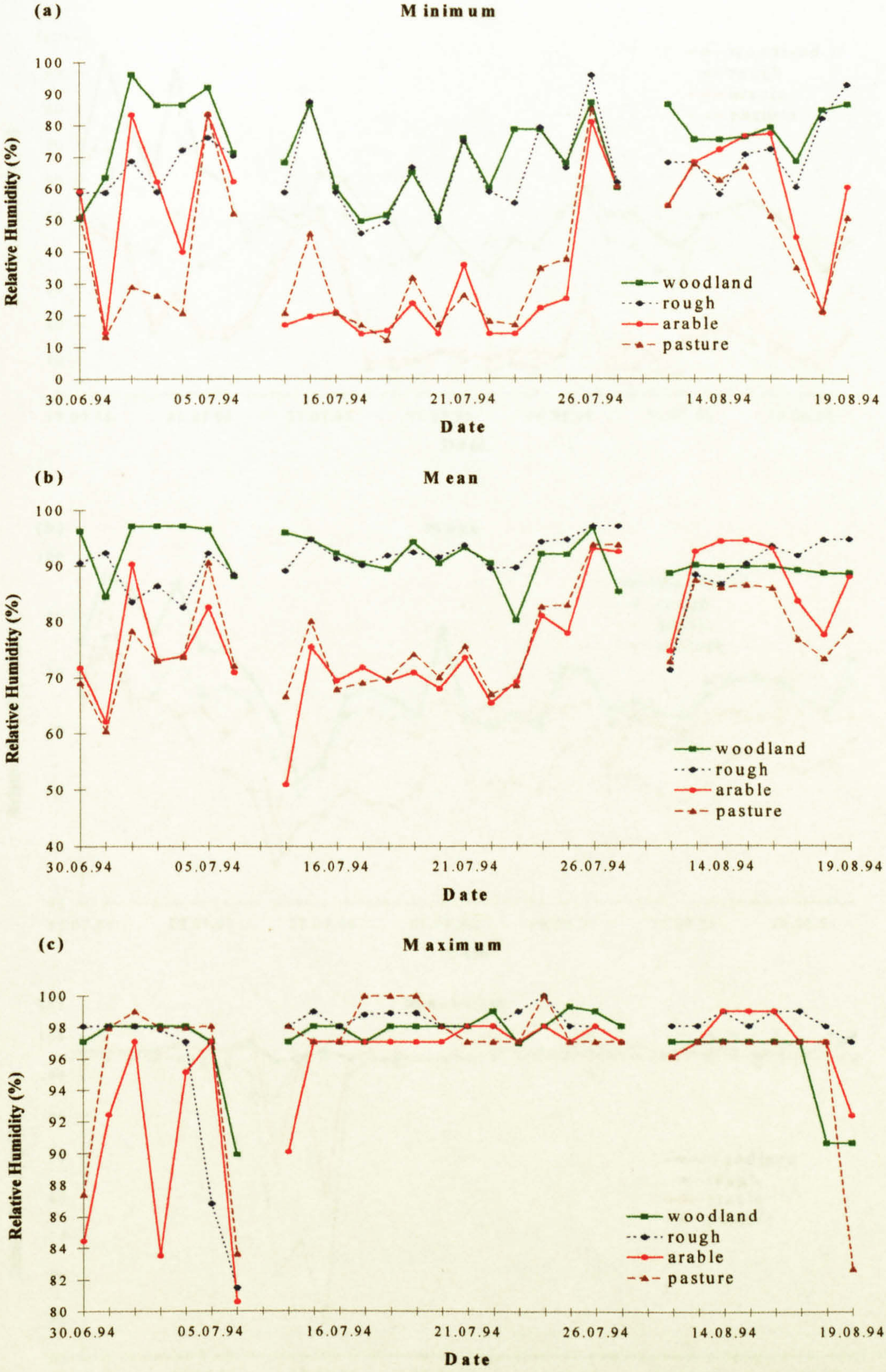
Daily minimum, mean and maximum humidity in 1994 and 1995 are shown in Figures 6.8, 6.9 respectively. Minimum relative humidity was diurnally variable especially in arable and pasture, daily maximum humidity was usually close to 100% and associated with dew. Humidity differed between years and radio-tracking habitats (Hotellings $T^2_{3,178}$=0.462, $T^2_{9,530}$=0.908 respectively $P_{(both)}$<0.001). Univariate post hoc tests revealed that mean and minimum humidity were significantly higher in 1994 than in 1995. ($F_{1,180}$=77.00, $F_{1,180}$=33.72 respectively, $P_{(both)}$<0.001). Mean and minimum humidity in woodland and rough grassland were significantly higher than those in arable and pasture ($F_{3,180}$=31.39, $F_{1,180}$=39.63 respectively, $P_{(both)}$<0.001).

(ii) Temperature

Daily minimum, mean and maximum temperatures in 1994 and 1995 are shown in figures 6.10 and 6.11 respectively. Maximum temperature was more variable than minimum and mean temperatures. July 1994 was warm and sunny producing high maximum temperatures. Cloud cover on 21st - 26th August raised minimum temperatures. July 1995 was hot and dry with high maximum and low minimum temperatures. Temperature differed between years and habitats (Hotellings $T^2_{3,178}$=0.659, $T^2_{9,530}$=0.927, respectively $P_{(both)}$<0.001). Univariate post hoc tests revealed that mean and maximum temperatures were significantly lower in 1994 than in 1995 ($F_{1,180}$=71.84, 53.73 respectively, $P_{(both)}$< 0.001). Mean and maximum temperatures in woodland and rough grassland were significantly lower than those in arable and pasture. Rough grassland had a significantly higher temperature than woodland. ($F_{3,180}$=16.90, 44.09 respectively, $P_{(both)}$ <0.001). Microclimate data from the four main radio-tracking enclosures are summarised in tables 4 - 7 of Appendix D.

Figure 6.8

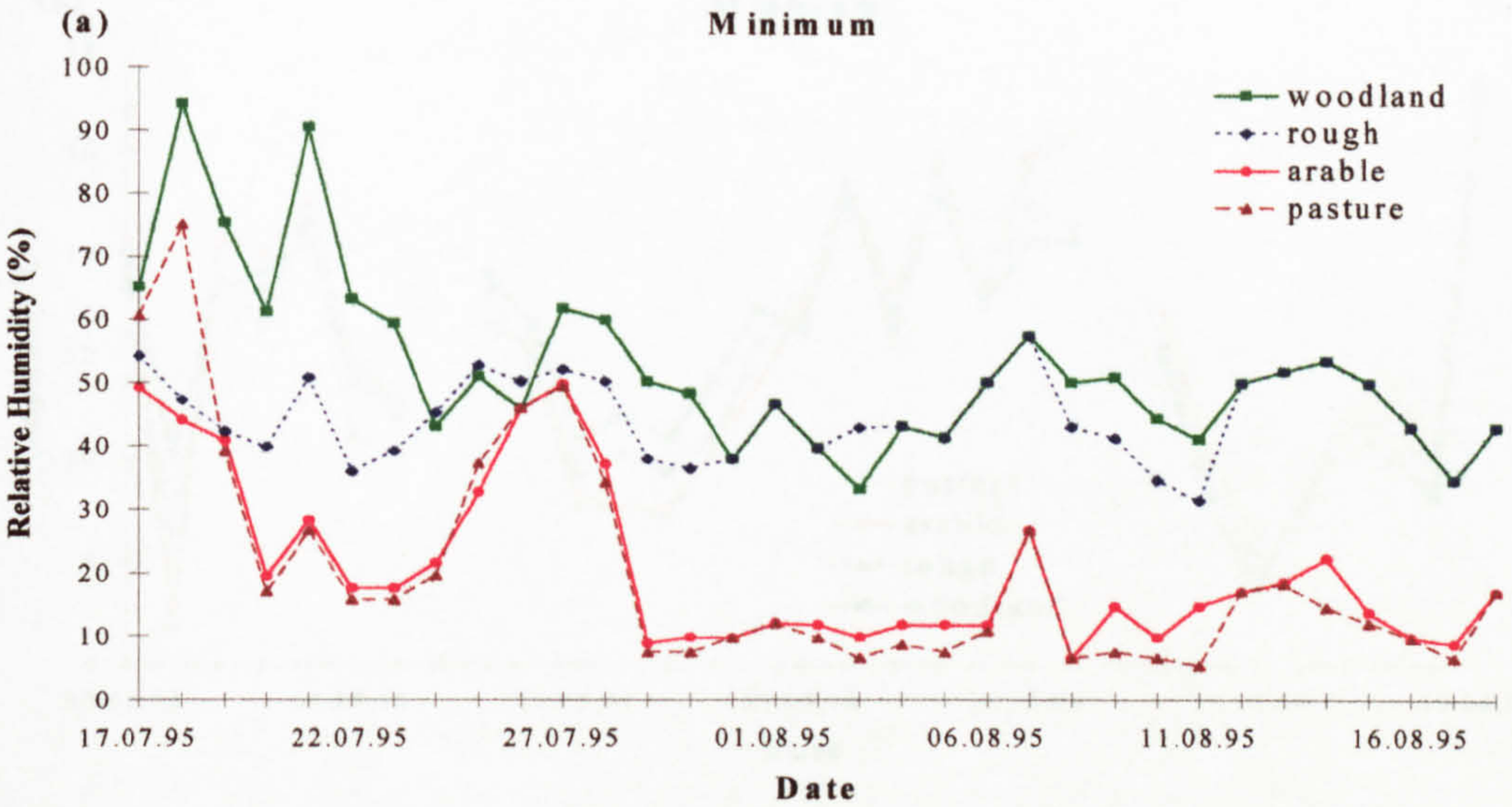
Relative Humidity At Ground Level In The Radio-tracking Area - 1994

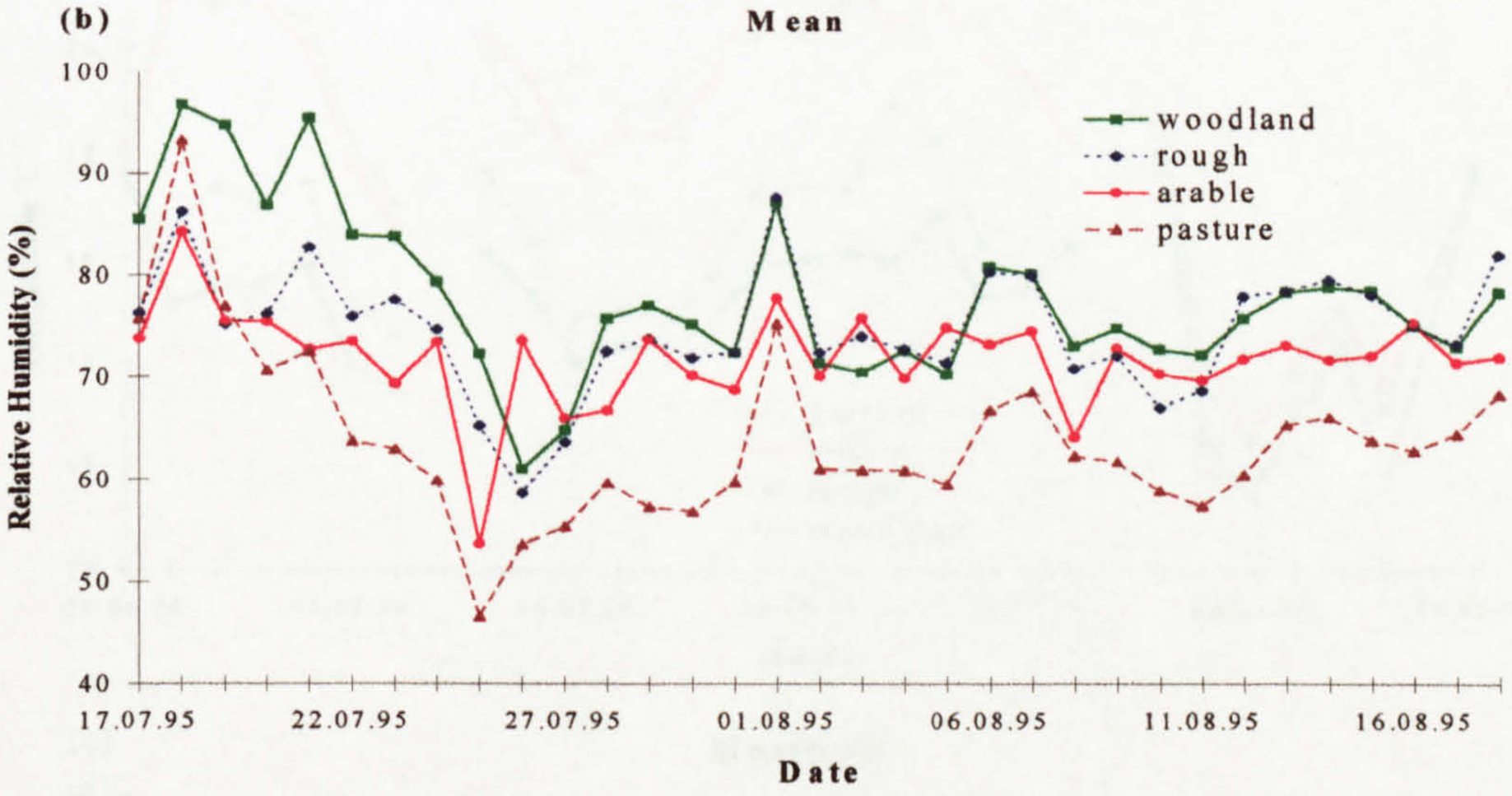


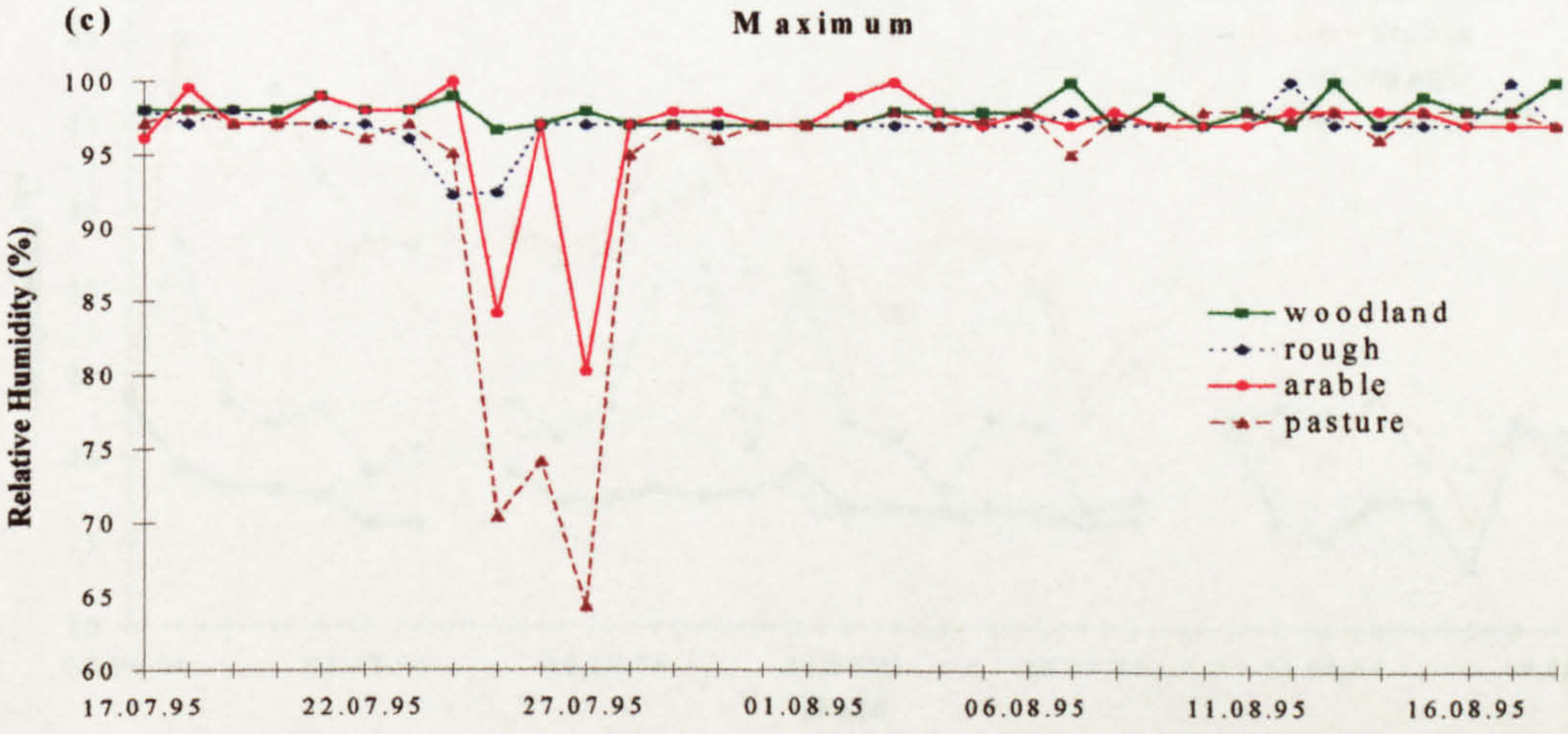
Humidity was measured using "Tinytalk" data loggers

Figure 6.9

Relative Humidity At Ground Level In The Radio-tracking Area-1995

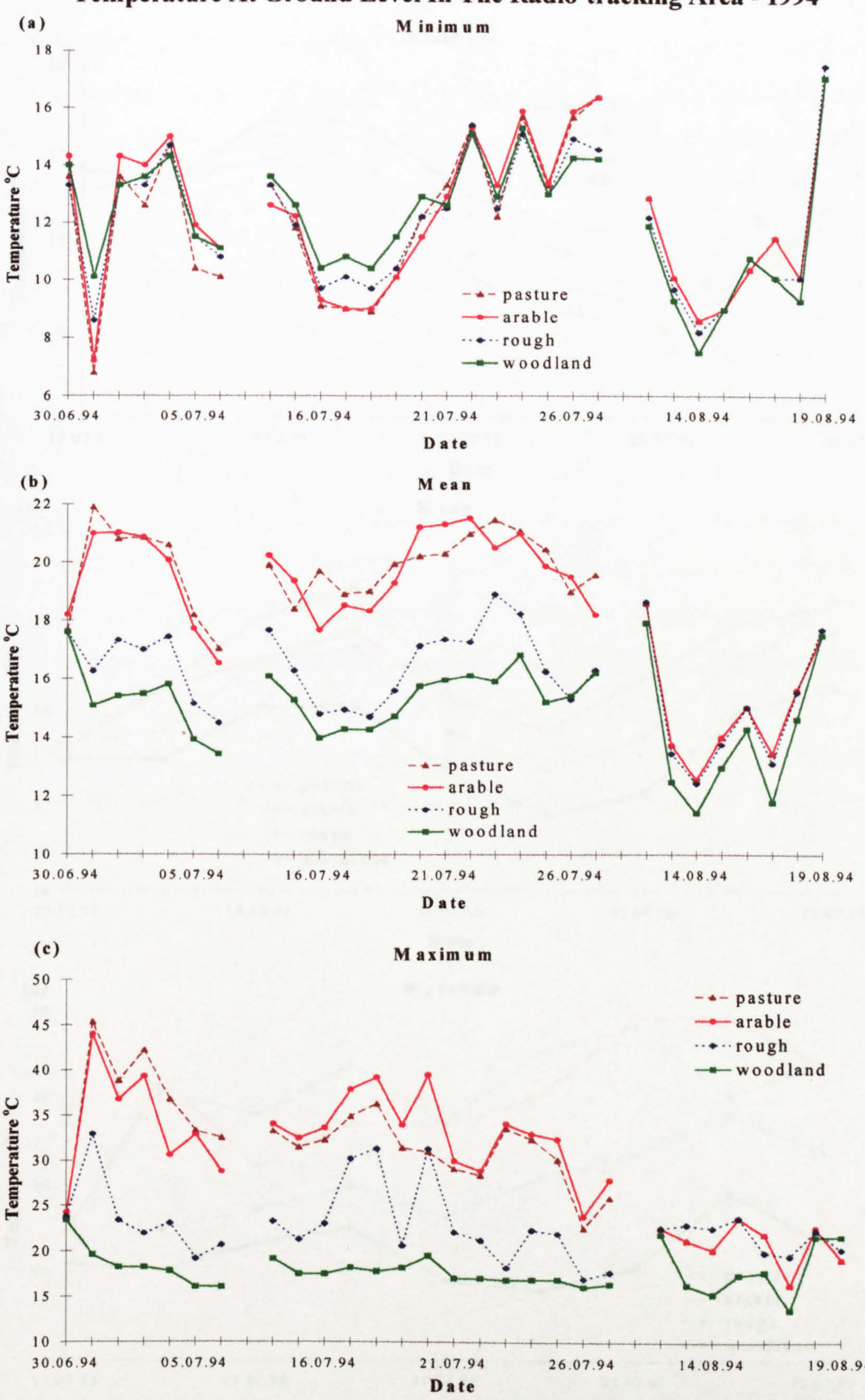






Humidity was measured using "Tinytalk" data loggers

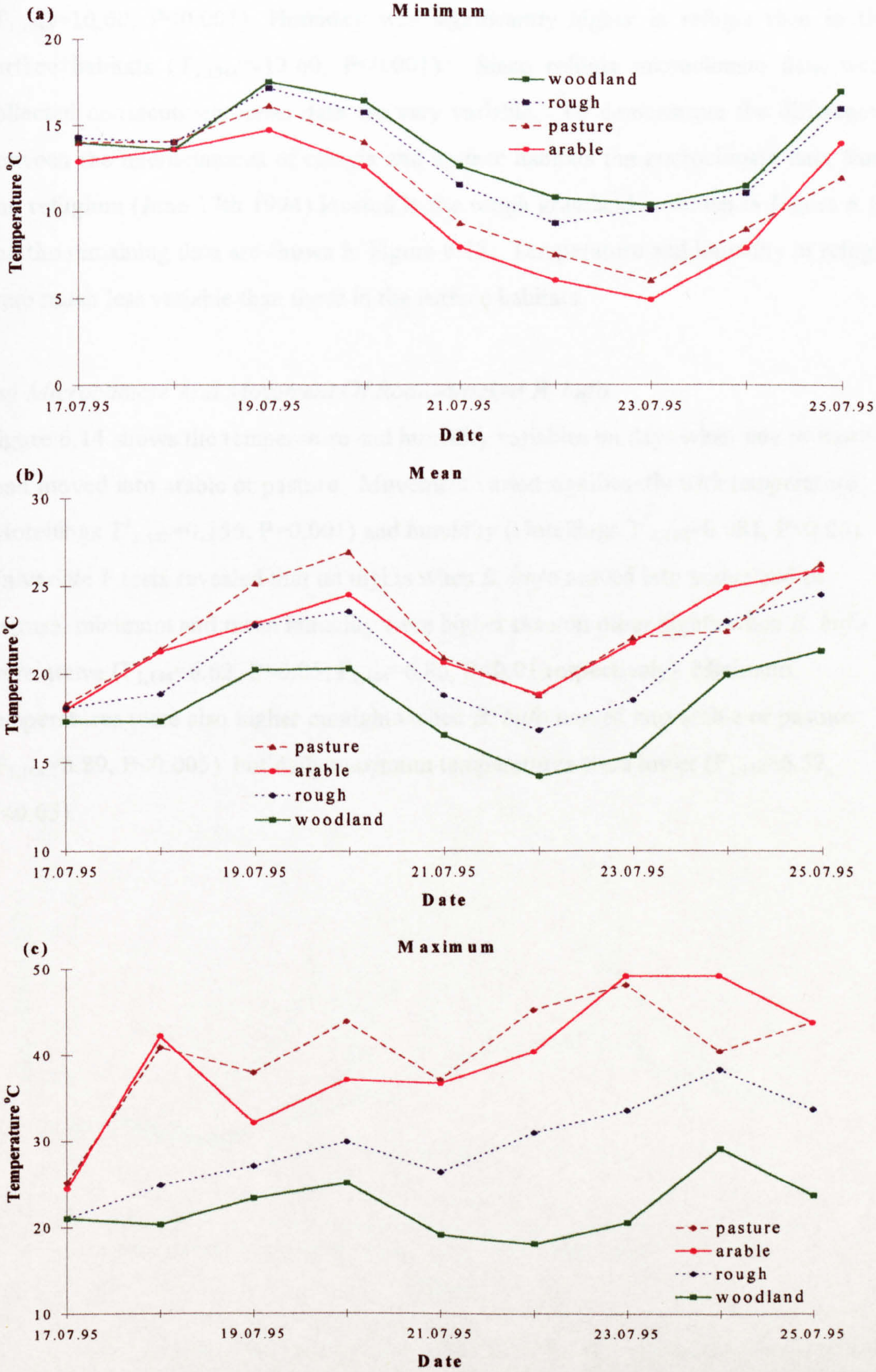
Figure 6.10



Temperature was measured using "*Tinytalk*" data loggers

Figure 6.11

Temperature At Ground Level In The Radio-tracking Area - 1995



Temperature was measured using "T*inytalk*" data loggers

(iii) Microclimates In Refugia And In Surface Habitats

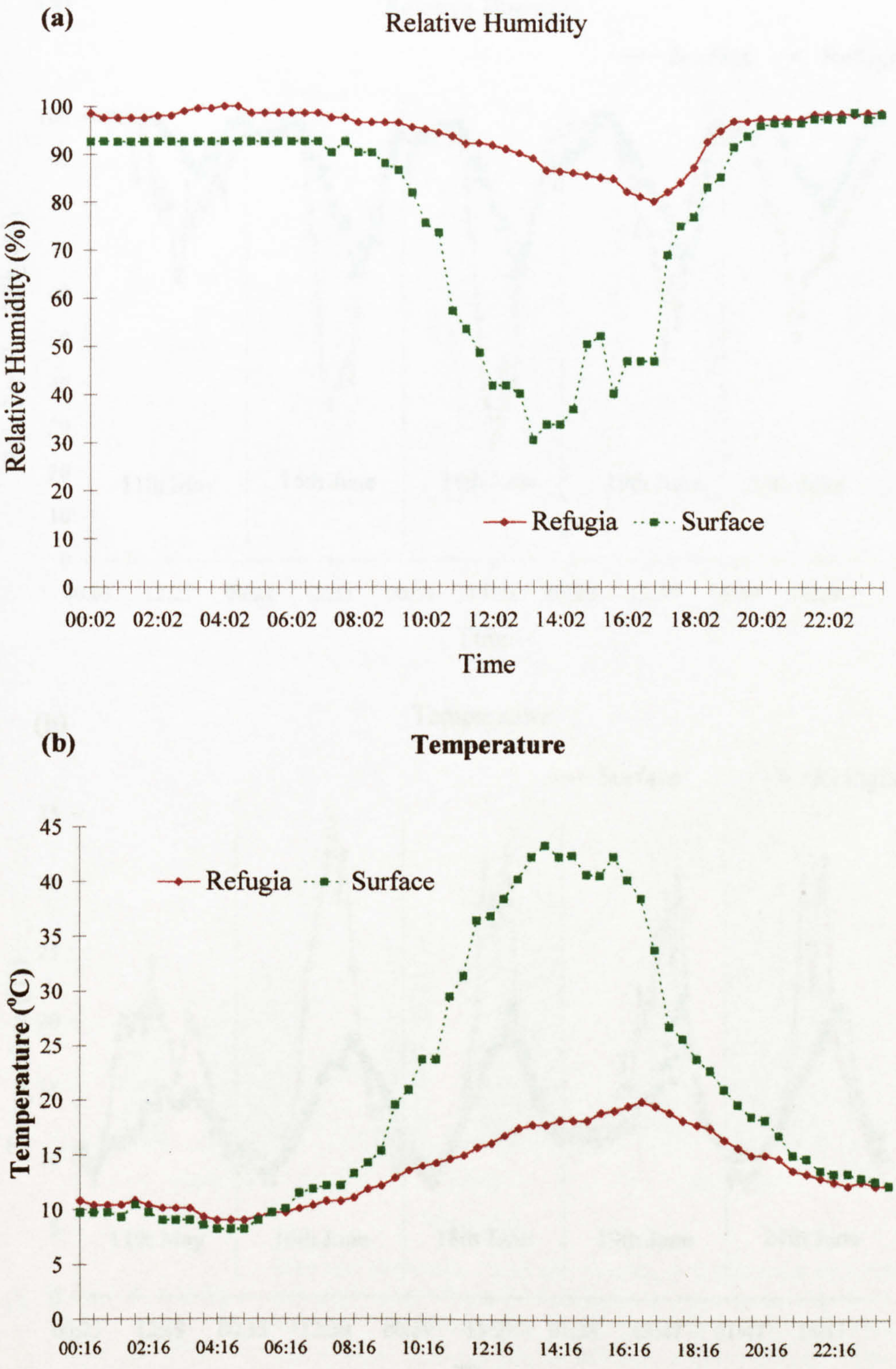
Temperatures in the surface habitats were significantly higher than those in refugia ($T_{1,1044}$=10.60, P<0.001). Humidity was significantly higher in refugia than in the surface habitats ($T_{1,1044}$=-13.60, P<0.001). Since refugia microclimate data were collected consecutively mean data are very variable. To demonstrate the differences between the microclimates of refugia and surface habitats the microclimate data from one refugium (June 17th 1994) located in the rough grassland is shown in Figure 6.12 and the remaining data are shown in Figure 6.13. Temperature and humidity in refugia were much less variable than those in the surface habitats.

(iv) Microclimate And Movement Of Radio-tracked B. bufo.

Figure 6.14 shows the temperature and humidity variables on days when one or more toad moved into arable or pasture. Movement varied significantly with temperature (Hotellings $T^2_{3,142}$=0.156, P=0.001) and humidity (Hotellings $T^2_{3,142}$=0.081, P<0.06). Univariate F tests revealed that on nights when *B. bufo* moved into arable and/or pasture, minimum and mean humidity were higher than on other nights when *B. bufo* were active ($F_{1,144}$=6.62, P<0.05; $F_{1,144}$=6.86, P<0.01 respectively). Minimum temperatures were also higher on nights when *B. bufo* moved into arable or pasture ($F_{1,144}$=8.89, P<0.005) but daily maximum temperatures were lower ($F_{1,144}$=6.52, P<0.05).

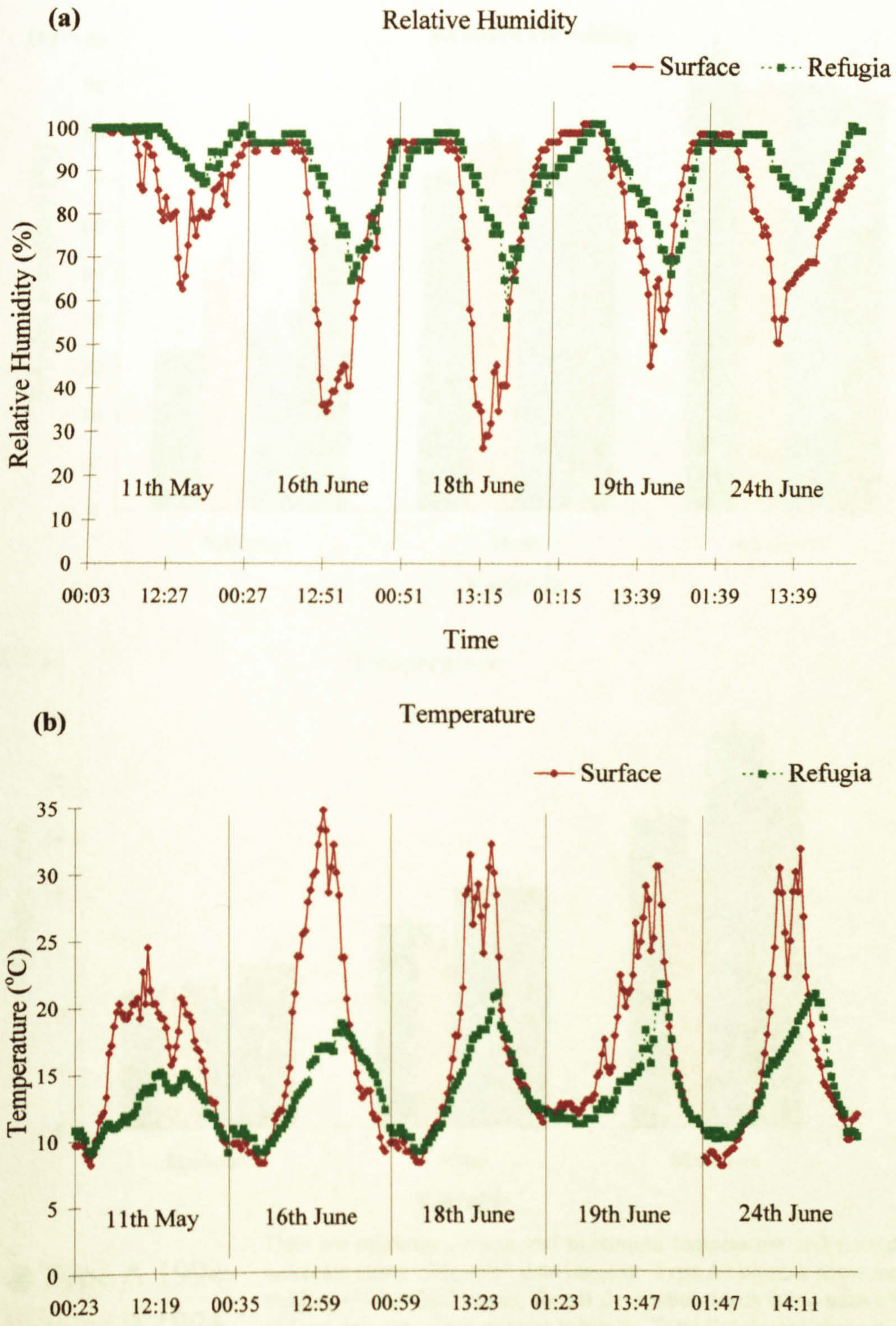
Figure 6.12

Relative Humidity and Temperature In Refugia and Surface Habitats -17th June 1995



A representative example of data collected used to show variability in refugia temperature and humidity compared with microclimate at the ground surface. Temperature and humidity were measured using "T*inytalk*" data loggers.

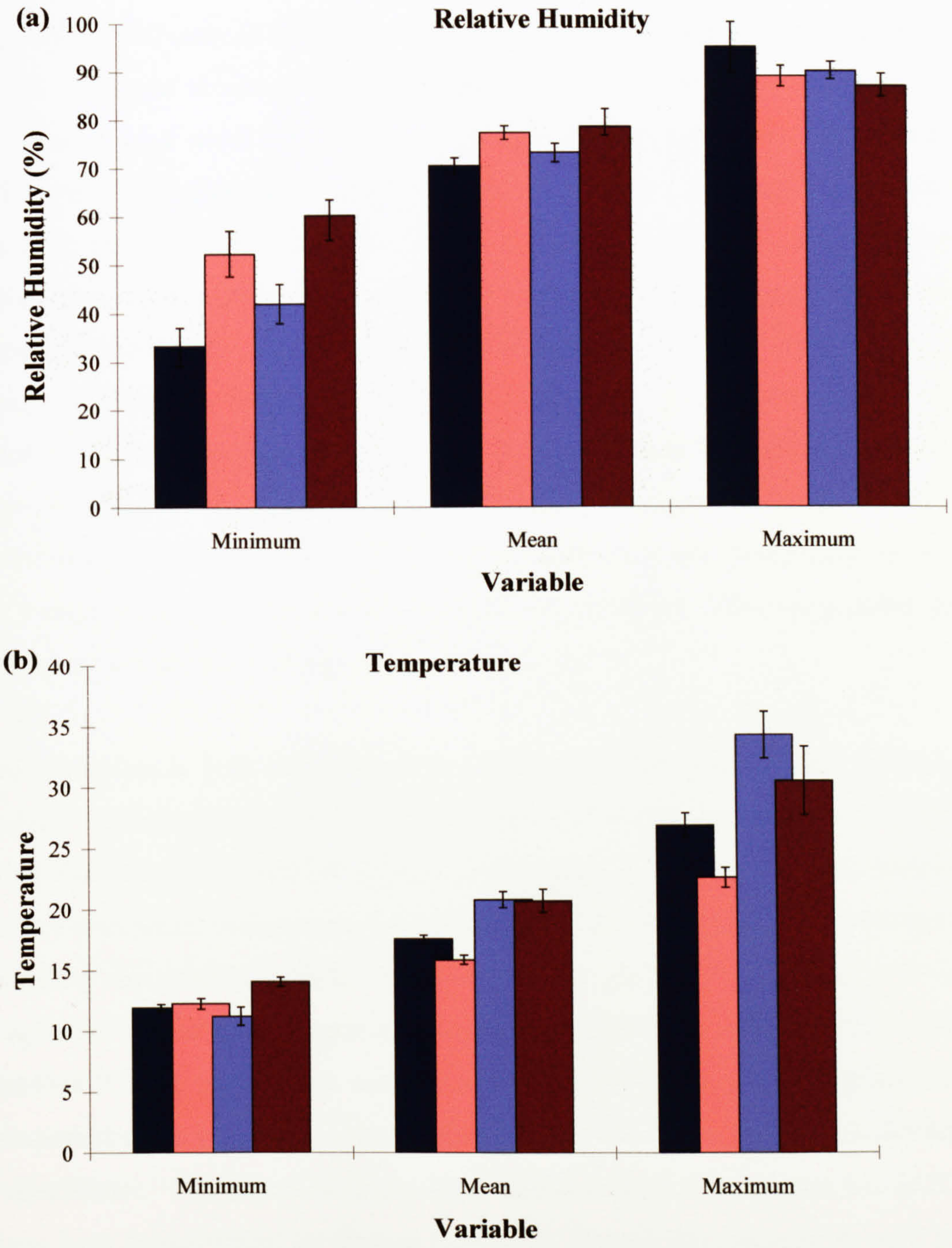
Figure 6.13
Relative Humidity and Temperature In Refugia and Surface Habitats



Temperature and humidity were measured using "T*inytalk*" data loggers. One humidity and one temperature logger were placed in each of the five refugia and another of each temperature and humidity loggers were placed on the surface immediately above the refugium.

Figure 6.14

Movement Of *B. bufo* and Climatic Variables



Data are minimum, mean and maximum temperature and humidity collected using "*Tinytalk*" data loggers. Type A columns show mean values, +/- standard errors, for all days when toads **were active** but did not enter arable or pasture habitats. Type B columns show mean values, +/- standard errors, for days when one or more animals moved into arable or pasture from preferred habitats. A day refers to the diurnal period preceding toad activity, and the period of toad activity. For details see text.

6.6 DISCUSSION

B. bufo demonstrated a high tolerance to dehydration, surviving losses of up to 58.6% of body mass. *B. bufo* survived body mass losses of up to 60% in a laboratory study by Rey in 1937 (quoted in Jorgensen 1997). Tolerance to high percentage loss of body water has been shown in other amphibia such as *Scaphiopus hammondii* a xeric adapted species which survived losses of over 46% of body mass (Thorson 1955). Thorson (1955) showed that small *B. bufo* had a higher percentage body water than large *B. bufo* and a greater surface area to body ratio; under identical conditions they lose water more rapidly. There was no correlation between mass loss and snout-vent length in the current study. The variables in enclosures are more difficult to regulate than the laboratory investigations of Rey and Thorson. Enclosures of $2m^2$ offer *B. bufo* an opportunity to locate some refuge from the ambient microclimate and this may vary with individual. Activity levels may also vary between individuals. Carey (1978) radio-tracked *B. boreas boreas* and *B. boreas halophilus* and found body temperature of a single animal can vary between 25-30°C over 24 hours. This was primarily due to nocturnal and diurnal activity.

Microclimates in both experimental enclosures and radio-tracking area differed with habitat. Short grassland was a good simulation of the improved pasture habitat with high diurnal microclimatic fluctuations. Woodland and rough grassland habitats (in both experimental enclosures and the radio-tracking area) ameliorated temperature and humidity fluctuations. These habitats provided shade from sunlight and rotting vegetation provided moisture at ground level and potential refugia. Pasture and arable habitats in these experiments and in the field are highly exposed and variable in both temperature and relative humidity, offering *B. bufo* little protection from the ambient microclimate. The effects of open habitat on the survival and moisture loss of *B. bufo* have been demonstrated by Denton (1991); he showed that common *B. bufo* kept in open dune habitat-specific enclosures died due to desiccation.

Refugia have two primary roles: protection from predation and prevention of desiccation. An amphibian that moves from a refuge may become exposed to high ambient temperatures and potentially desiccating conditions. The refugia in the current

study provided substantial amelioration of microclimates found in woodland and rough grassland, which in turn were less desiccating than pasture and arable habitats (Figures 6.12, 6.13). Dobkin *et al* (1989) radio-tracked *B. marinus* using calibrated transmitters and found that body temperatures of hiding *B. bufo* reflected those of their refugia, and were more stable than those of active animals.

The results of the enclosure experiments suggest that humidity is of primary importance in preventing desiccation. In the experimental enclosures minimum humidity correlated with mass loss but mean, minimum and maximum temperature did not (Figure 6.6). The importance of humidity is also suggested by the radio-tracking data. The preferred habitats of radio-tracked *B. bufo* (chapter 3) were woodland and rough grassland. These two habitats were significantly more humid than the least used habitats - pasture and arable (Figures 6.8, 6.9). The importance of humidity in amphibian water economy has been known since 1824 (Edwards, quoted in Jorgensen 1997). Edwards exposed frogs to air of 100% relative humidity producing mass losses of 0.25% per hour. When air humidity was reduced to between 54 and 58% anuran mass loss was increased to 1-2% per hour.

Temperature variables were also of potential importance in habitat preference. In the radio-tracking enclosure the preferred habitats (rough grassland and woodland) had lower mean and maximum temperature than pasture and arable (Figures 6.10, 6.11). Minimum temperatures were higher in woodland and rough grassland than in arable and pasture (Figures 6.10, 6.11). Diurnal temperatures were reduced by shading of dense vegetation in woodland and rough grassland. At night the reverse occurred and heat was prevented from radiating by the vegetation reducing the nocturnal temperature decrease.

Activity of amphibians, including habitat preference, can be influenced by microclimate. In an Israeli population of the terrestrial *Salamandra salamandra*, activity was closely associated with rain or humidity levels; animals were rarely active at a relative humidity of 60% or less (Degani and Warburg 1978). Cloud cover in summer can produce low maximum and high minimum temperatures. Cloud cover

during the day reduces sunshine and therefore reduces the maximum temperature. At night cloud cover can prevent/reduce radiation of heat into the atmosphere. Since minimum diel temperatures in the current study, occurred at night cloud cover at night tended to raise the minimum temperature.

The habitat preference of animals in the current study was influenced by microclimate; Radio-tracked *B. bufo* only moved into the least preferred habitats (arable and pasture) when humidity and daily minimum temperatures were high. Movement into these less preferred habitats was also correlated with low maximum temperatures. Thus movement into arable and pasture occurred when conditions were cloudy or overcast. Many other workers have confirmed the importance of climate on anurans but most concentrate on activity threshold limits or on numbers of animals active. For example the number of frogs (*Leiopelma archeyi*) emerging from forest floor retreats was strongly correlated with relative humidity, rainfall and wetness of vegetation (Cree 1989). Grist (1994) tried to assess the interdependence of climate variability, habitat and the activity of *B. bufo* and *B. calamita.* He found common toad captures showed the greatest correlation with site but numbers of active animals were also correlated with temperature and sunshine variables.

The habitat preferences of *B. bufo* differed significantly between the two years as did the microclimates of these habitats. The radio-tracking enclosure was cooler and more humid in 1994. In this year *B. bufo*, especially males, showed significantly more movement into arable and pasture habitats (chapter 3). Alteration of habitat selection may be the initial response of *B. bufo* to a change in climatic conditions and may be linked to toad fitness. Reading and Clarke (1995) compared changes in the body condition of *B. bufo* with toad density, rainfall and temperature in the previous summer for 10 years from 1983-1993. They correlated an observed decline in body condition with toad density and climatic temperature.

Significant correlations, no matter how strong cannot establish a causal connection. Other factors may be influencing the observed response in the current study. For example behaviour of invertebrates, and thus toad food availability is linked to

microclimate. The interplay between microclimate, food and habitat are discussed in Chapter 8. Despite these caveats the replication of results in the two enclosure experiments and the radio-tracking area is strong corroboration for the influence of microclimate, and relative humidity, in particular on habitat preference.

(B) PREDATION

6.7 INTRODUCTION

Anurans are usually central in community food webs often functioning as arthropod predators (e.g.Toft 1980, 1985) and as prey to both vertebrate and invertebrate predators (e.g. Denton and Beebee 1997). Predation of anuran larvae is well documented (e.g. Anholt and Werner 1995, Kupferberg 1997). Predation of larvae is a key factor in the population dynamics of many species (e.g. Denton and Beebee 1997). Predator avoidance tactics by anuran larvae are diverse and well studied. Many species of larvae show reduced activity in the presence of predators (e.g. Kupferberg 1997). Larvae of some species, including *B. boreas*, show an alarm response to chemical cues from damaged conspecifics (e.g. Hews and Blaustein 1985). Some anuran species show predator-induced phenotypes. For example larvae *of Hyla chrysoscelis* show reduced activity, brighter and more colourful tail fins in the presence of predatory dragon-fly larvae (*Anax junius*). These anti-predator strategies are induced as they produce reduced survival in the absence of predators (McCollum and Vanbuskirk 1996).

Most species of bufonids have epithelial skin toxins in the larval stage. Species with toxic skin (including *B. bufo*) are often darkly coloured and form obvious aggregations which may be aposematic (Peterson and Blaustein 1991). Distasteful skin reduces larval predation from vertebrate predators however protection varies between predator species. For example Denton and Beebee 1997 studied predation of *B. calamita* in semi-natural replicated ponds by invertebrate and vertebrate predators. Rudd (*Scardinius erythrophthalamus*) was undeterred by tadpoles skin toxins and consumed toad larvae and invertebrates almost indiscriminately. Whereas Carp (*Cyprinus carpio*) and perch (*Perca fluviatilis*) eliminated invertebrate predators leaving tadpoles unpredated. Distasteful skin seems to offer little protection from invertebrates however their is larger larvae may be to large for invertebrates and thus there is a size refuge against invertebrate predators (e.g. Denton and Beebee 1997, Travis *et al* 1985, Heyer and Muedeking 1976).

Predation of non-larval stages of terrestrial anurans is less well documented and is most likely to occur in the breeding season when anurans are mobile and out of refugia which increases their visibility. Adult *B. bufo* have been predated by a range of mustelid species during the breeding season (Weber 1990, Sidorovich and Pikulik 1997). Ranids were preferred over *B. bufo* and *B. viridis* by the semi-aquatic mustelid predators due to the presence of skin toxins in the bufonids (Sidorovich and Pikulik 1997).

Outside the breeding season, refugia will provide protection from visual predators however olfactory predators such as grass snakes (*Natrix natrix*) are able to locate *B. bufo* when in refugia (Denton and Beebee 1993). *Bufo* species may show antipredator responses for example post-metamorphic juveniles are able to avoid chemical cues from species of sympatric predatory snakes in laboratory experiments (Flowers and Graves 1997).

6.8 RATIONALE AND AIMS

Predator numbers and species vary between habitats and different habitats may offer different protection from predation. The presence of a predator may alter the behaviour of the prey including habitat selection. The first aim was to identify the predators of *B. bufo* in Leicestershire by observation of corpses during the breeding season. The second aim was to compare the predation risks in different habitats. Predation risk was assessed in terms of visibility of *B. bufo* in different habitat specific enclosures.

6.9 METHODS

During the breeding seasons of 1994 and 1995 population counts of 26 *B. bufo* breeding sites were conducted. The sites and population estimation are described in chapter 7. Dead adults seen on circuits of the breeding sites were counted and the cause of mortality was assigned into one of four categories; traffic casualties, bird predation, mammalian predation and other mortality. Traffic casualties were obvious as they were crushed and on the road. Bird predation was identifiable by the peck marks usually on the skull or back of the corpse. Mammalian predation was identified

by faeces, tracks and bite marks on corpses. At some sites mammalian and bird predation were identified by direct observation of the predators. The estimates of both bird and mammalian predation would be underscored as some animals would be removed or taken whole by birds or mammals. The other mortality category included all cases where cause of mortality was uncertain and those cases due to fatigue, stress or parasitism.

Up to ten sites each night were visited on a three night rotation and all sites were visited at least three times during the breeding season. Some experimental sites were visited more frequently making a total of 85 site visits in 1994 and 96 in 1995. The frequency of mortality was recorded as the number of site visits where a category of mortality was observed divided by the total number of site visits that year. The abundance of mortality was the number of individuals in each category divided by the total number of dead individuals recorded.

The predation risk in different habitats was compared by recording the mortality of radio-tracked individuals which was described in chapter 3. The visibility in different habitats was assessed using the experimental enclosures described in section A, 6.4.2 Two experiments were conducted one in May (25th-31st) and one in June (21st-27th), 1995. Two hours before counting Dr. R. S. Oldham placed between five and ten animals in each enclosure to ensure that the number of animals in each enclosure was not known by L. Bardsley prior to counting. Each of the enclosures was visited at dusk on four occasions during each of the two experiments. Each enclosure was circled for one minute using a red filtered torch and the number of *B. bufo* seen was recorded. The order in which the cages were counted was randomised each night to avoid any effects of poor initial search image. Visibility indices (V) were calculated for each of the replicate enclosures. The number of *B. bufo* seen on each visit was divided by the total number left alive in the enclsoures to produce proportional visibility on each night. The mean of the four proportional visibility measurements in May was used as the visibility index (V). The visibility index was calculated in the same manner for the experiment in June. A Wilcoxon Matched pairs compared the visibility indices per replicate enclosure between May and June. Since there was no significant difference

between the two months, data were used as replicates for further analysis. Kruskal-Wallis one-way non-parametric ANOVA compared visibility of *B. bufo* with habitat as the independent variable.

6.10 RESULTS

The total number of dead *B. bufo* observed was 207 in 1994 and 108 in 1995. The percentage frequency and abundance of mortality for both 1994 and 1995 are shown in Table 6.2. In both 1994 and 1995 the highest percentage mortality at breeding sites was due to road casualties. Predation by birds was observed more frequently and more abundantly than predation by mammals.

In the radio-tracking enclosure (Chapter 3) a total of seven of the 70 radio-tracked animals were killed by predation; five in 1994 and two in 1995. Five of the predated radio-tracked animals were taken by birds. All animals predated were out of refugia and above ground when corpses were detected.

Table 6.2

Observed Mortality at 26 Breeding Sites

	YEAR	Mortality			
		Road Casualties	Mammalian Predators	Avian Predators	Other Mortality
Frequency of Mortality (% of maximum)	1994	46.88	4.17	15.63	3.13
	1995	23.53	4.71	14.11	1.18
Number of Animals (% of Total)	1994	68.12	2.90	19.81	9.18
	1995	73.15	5.56	18.52	2.78

Visibility of *B. bufo* did not differ between the May and June experiments ($Z_{2,63}$ = -0.140, P = 0.888). Visibility did differ between habitats ($\chi^2_{2,63}$=15.1579; P=0.0005). The mean visibility indices per three replicate enclosures are shown in Table 6.3. Mean visibility indices were highest in short grassland enclosures in June (0.812+/-0.016) and lowest in long grassland enclosures in the same month (0.065+/-0.033). *B. bufo* were only located in the long grassland if they moved.

Table 6.3

Visibility In Different Habitats

	HABITAT		
MONTH	Woodland	Long Grassland	Short Grassland
May	0.265 (0.008)	0.139 (0.042)	0.740 (0.008)
June	0.259 (0.019)	0.065 (0.033)	0.812 (0.016)

Values are the mean of visibility index from 3 replicate enclosures. Values in Brackets are the standard error of the mean.

6.11 DISCUSSION

The importance of predation on adult *B. bufo* in population dynamics of this species requires further study. The highest observed mortality in both years was from road casualties. Road casualties were more visible and more likely to be observed during pond visits than any other form of mortality. However road casualties of *B. bufo* are high when seasonal migrations occur across roads (e.g. van Gelder *et al* 1986a, Oldham and Swan 1991). Fahrig *et al* (1995) reported that traffic mortality had a significant effect on the local density of anurans. Records of mammalian and avian predation of adult *B. bufo* are limited. Mustelid predation of *B. bufo* has been recorded (Sidorvich and Pikulik 1997) however few of the mustelid species listed as major amphibian predators are present in the Leicestershire area. Since mammalian predation was the hardest to identify with certainty and some mammals may eat *B. bufo* whole, this category was the most likely to be underscored. Avian predators may also remove the corpse whole from the breeding site so avian predation may also be underscored. Avian predation was more frequent and more commonly occurring than mammalian predation probably due to the higher number of avian predators in the study areas.

The most preferred habitat by radio-tracked *B. bufo* was the long grassland which had the lowest visibility index (Table 6.3) and would offer more protection from predation. Predation in the radio-tracking enclosure was rare, only a total of seven animals out of 70 tracked in 1994 and 1995. This may be due to absence of predators in the radio-tacking area, however birds, in particular crows and magpies were common in the

surrounding area. It is more likely that refugia protected *B. bufo* from diurnal visual predators. Evidence that refugia do not protect *B. bufo* from olfactory predators comes from Denton and Beebee (1993). Grass snakes (*Natrix natrix*) ate three out of eight radio-tracked *B. bufo* whilst in their refugia. No grass snakes were found within the radio-tracking area. The present investigation into predation was limited, and much more work is still required to establish the influence of predation on habitat selection. However in the current study *B. bufo* were quantifiably more visible in open grassland and therefore vulnerable to avian predation.

6.12 CONCLUSIONS

- *B. bufo* demonstrated a high tolerance to dehydration, surviving losses of up to 58.6% of body mass.
- Mass loss of *B. bufo* was significantly higher in short grassland enclosures than in woodland or long grassland enclosures.
- Mass loss of *B. bufo* in different habitats was not correlated with length of animal but was correlated with minimum humidity.
- Microclimates in experimental enclosures and radio-tracking area varied between habitats. Woodland and long grassland were significantly more humid and had lower maximum and higher minimum temperatures than arable and pasture.
- Microclimates of refugia had smaller diurnal variations in temperature and humidity than the microclimates of surface habitats.
- *B. bufo* were significantly more visible in open habitats than in woodland and rough grassland.

7: HABITAT AVAILABILITY AND POPULATION SIZE

7.1 INTRODUCTION

Estimation of animal population size is a fundamental part of biology. When animals are small, difficult to locate and cryptically coloured, such as small mammals and most European anurans, absolute counts of population sizes are not possible (Sutherland 1996). These taxa therefore require estimation of populations size from a model or by use of a comparative population index (Southwood 1978, Sutherland 1996). Population studies can be divided into two types: extensive and intensive (White and Garrot 1990). Extensive types are carried out over a large area and are normally related to the distribution of a species. In these studies each site is sampled on a few occasions and the population data obtained can be related to edaphic, vegetative or climatic features. Intensive studies involve the continual observation of one population over an extended period.

Most studies on amphibians, and anurans in particular, are intensive and concentrated on the breeding site. Many temperate zone anuran species, especially ranid and bufonids migrate *en masse* to the breeding site at the onset of the breeding season. This breeding migration provides an ideal opportunity to study the breeding population of amphibia in a short period of time. These intensive studies usually concentrated on one site and determined the population size and characteristics of a single species of amphibian (e.g. Frazer 1966, Gittins *et al* 1980, Hoglund and Robertson 1987, Arntzen and Teunis 1993). Most of these intensive studies have characterised the breeding migrations and behaviour of *B. bufo* and have found that males arrive earlier and stay longer at the breeding site than females (e.g. Moore 1954, Frazer 1966, Gittins *et al* 1980, Wisniewski *et al* 1980, Reading and Clarke 1983, Hoglund and Robertson 1987). The earlier arrival, longer stay at the breeding site by males and the later sexual maturation of females (e.g. Hemelaar 1983) have contributed to lower numbers of males than females observed at many breeding sites (e.g. Frazer 1966 Gittins *et al* 1980, Wisniewski *et al* 1980, Reading and Clarke 1983, Hoglund and Robertson 1987). The breeding migration and behaviour *of B. bufo* have been described more fully in Chapter 1.

Most extensive surveys of amphibians have looked at the presence or absence of amphibian species and have related presence or absence to different variables. Surveys have looked at the presence and absence of amphibian species in gardens (Mathias 1975, Beebee 1979, Banks and Laverick 1986) in agricultural lowlands (Beebee 1981) and in chalk uplands (Beebee 1977b). Other surveys have compared changes in the numbers of amphibian breeding sites with time (Cooke 1972, Cooke and Ferguson 1974, Bell 1970). The final group of studies have compared habitat variables with amphibian species richness (Laan and Verboom 1990, McCoy and Mishinsky 1994). In general these studies have found a decline in the number of amphibian breeding sites and species richness since the turn of the century.

A combination of both extensive presence or absence studies and an intensive single site study on *B. bufo* was published by Oldham and Swan (1991). This compared the results of a questionnaire survey of the whole country with results of mark and recapture studies of a Leicestershire breeding site. The results of this study confirm the loss of amphibian breeding sites during the later half of the 20th Century. Methods of conservation including tunnel systems and translocation to combat this trend are also described in this study.

An extensive study comparing breeding population size of *B. bufo* with a wide range of variables was published by Cooke in 1975. This study used survey data from a combination of breeding site data bases and questionnaires, to obtain data on both amphibian population size and the habitat variables. The habitat variables measured were mainly aquatic though some consideration was given to land use around the pond. The Cooke study found that *B. bufo* tended to form large breeding colonies in deeper water than the sites chosen by *R. temporaria*. *B. bufo* were less likely to be found in garden and arable habitats than *R. temporaria* (Cooke 1975).

Methods used to study the population size and ecology of *B.bufo* in small numbers of ponds have included perimeter fencing, pitfall trapping and marking (e.g. Gittins 1983, Reading and Clarke 1983, Reading 1988, Oldham and Swan 1991) repeated perimeter searches (e.g. Gittins *et al* 1980, Wisniewski *et al* 1981), mark release and recapture

(e.g. Frazer 1966, Wisniewski *et al* 1980) and counting number of *B. bufo* observed (e.g. Hoglund and Robertson 1987). Perimeter fencing and mark and recapture methods require abundant resources of time and material, therefore are only feasible for small numbers of sites. Estimates of population size at large numbers of ponds (>20) can be obtained by direct counting of *B. bufo* however this method is also very time consuming.

Radio-tracked *B. bufo* demonstrated strong terrestrial habitat preference (chapter 3). Dietary analysis (chapter 5), invertebrate sampling (chapter 4) and microclimate measurements (chapter 6) suggest that habitat choice will influence both the food gain and survival of adult *B. bufo*. The aim of this chapter was to determine the habitat correlates of the population size of *B. bufo* from breeding sites in NW Leicestershire. Population size was estimated by direct population counts at a range of sites. The accuracy of population estimates was evaluated by an intensive trapping and mark recapture study of one breeding site. The terrestrial habitat around each breeding site was assessed in terms of percentage area of different habitat categories.

7.2 METHODS

7.2.1 Choice Of Study Sites

Populations were chosen from the same restricted geographical location (20Km^2) and were therefore subject to the same general climatic influences. Variation between populations at these sites was considered a response to habitat (both terrestrial and aquatic) variability. The study sites chosen ranged from small farm ponds to a disused reservoir. Ponds were chosen from a data base to give a range of terrestrial habitats and a concomitantly broad population range. All sites had the following properties:

(i) Ponds were able to support metamorphosis of *B. bufo* and metamorphosis had been recorded previously at each site.

(ii) Ponds were accessible and all or most of the perimeter could be traversed to facilitate counting.

(iii) Ponds were at least 1Km from the nearest water body which was known to produce metamorphs. This was to minimise the competition for the terrestrial

habitat from populations not within the study. Reading *et al* (1991) showed no interaction between *B. bufo* populations if the ponds were greater than 830m apart.

7.2.2 Population Indices

An estimate of population size at the thirty-two sites was made by night counting in the springs of 1994 and 1995. To do this a circuit was made of each breeding pond at night (at approximately 60m per minute) and the number of *B. bufo* present was recorded by a direct head count. *B. bufo* in the water and those within 1 metre of the waters edge were recorded. *B. bufo* on paths migrating to and from the pond were not used in further analysis. Ten sites were counted per night on a three night rotation. To ensure that the peak of the breeding season was counted, each site was visited on at least four occasions. The number of *B. bufo* seen at peak counts at each site depended upon:

(i) the number of *B. bufo* present

(ii) prevailing weather conditions

(iii) clarity of the water

The number of *B. bufo* present was the variable to be measured in this study and the prevailing weather conditions should be similar at sites within the same geographical location though microclimates will vary. Clarity of water did vary between sites. Sites where the bottom could be seen were termed clear water sites. At each of the 20 clear water sites numbers seen at and below the water surface were counted separately. The mean percentage of *B. bufo* at the water surface at all clear water sites was calculated at the time of the maximum counts obtained in each year. The mean percentage of animals at the water surface was used to correct the peak counts of the 6 turbid water sites in the corresponding year using the calculation shown below.

$$N_{(corr)} = \frac{100}{\overline{P}_{aw}} \times N_{(count)}$$

Where $N_{(Corr)}$ is the corrected value, $N_{(count)}$ is the peak value at each site and $\overline{P}_{aw}$ is the mean percentage of animals at the water surface.

As described in section 7.1, female *B. bufo* are usually less numerous and remain at the breeding site for a shorter period than males (e.g. Davies and Halliday 1977, Gittins *et al* 1980, Reading 1983). The terrestrial habitat preferences of males and females were shown to differ significantly in chapter 3. Habitat correlates of males and females may differ, therefore male and female *B. bufo* were counted separately. To confirm the status of all ponds as breeding ponds, each site was searched for tadpoles and/or metamorphs throughout June, July and August of each study year. Only sites with tadpoles/metamorphs were used in further analysis.

7.2.3 Evaluation Of The Population Indices

In 1995 an experiment was conducted to test the accuracy of the night counts i.e. what percentage of the *B. bufo* visiting a site were represented by the peak counts? Since females remain at the breeding sites for less time than males, they are less likely to be included in the peak counts (e.g. Frazer 1966, Bell 1970). Cooke (1975) states that the peak count of adult males can be doubled to give an estimate of the total number of animals present at the breeding site. To test this theory and to compare observed with actual sex ratios, one breeding pond was surrounded by a perimeter fence. This pond was chosen as it was privately owned reducing the risk of vandalism. The breeding population at this site was intermediate in size between small farm pond populations and large populations in reservoirs. The pond was situated in a large rural garden adjacent to a farm. The surrounding habitats were varied and included: rough grassland, short grassland and woodland.

The chicken wire perimeter fence (mesh size 1cm) was dug into the ground 3-5m from the waters edge and pitfall traps were dug on the interior and exterior of the fence to catch *B. bufo* leaving and entering the pond. All animals entering the pond were given a toe-clip which was specific to the direction from which the animal came. They were then placed over the fence and given all day to mix with the population in the water. Each night a count of the animals in the water was taken by circuiting the pond once and counting all *B. bufo* seen.

The perimeter fence was erected on the 25th March 1995 and some animals could have migrated before this time. Some animals would already inhabit the area of grassland within the enclosure and very small males may be able to get through the holes in the chicken wire and others may be able to climb over the fence. To assess what percentage were unmarked, and therefore not captured by the perimeter fence, 100 males and all females seen were captured on three separate occasions: 4th, 6th and 7th of April. On these evenings the numbers of marked animals were recorded and the percentage of unmarked animals of each sex was recorded.

7.2.4 Habitat Assessment

A 1Km radius surrounding each breeding pond was surveyed for habitat use. *B. bufo* have been shown to migrate at least this far by Gittins *et al* (1980). *B. bufo* have been shown to migrate further than 1Km for example Frazer (1966) found a marked *B. bufo* 1.25 miles from the centre of the breeding site. The 1Km radius was chosen as this was considered the maximum area available to the majority of *B. bufo* from each breeding colony, based on unpublished work of Dr. R.S. Oldham and Dr. D. Latham. The habitats were surveyed in Autumn 1994. A phase I NVC survey was initially applied by walking the area and recording habitats on Ordnance Survey (OS) maps (1:10,000 scale). The description of the Phase I survey methods are given in Appendix E. The habitat categories shown in Table E1 (Appendix E) were then summed to give the categories shown in Table 7.1.

These categories were chosen to give a limited number of variables for statistical reasons and for ease of comparison with other studies. These land cover categories were also chosen so that comparisons with the radio-tracking data could be made. The areas of each of these categories were measured from the annotated OS maps using a planimeter

Table 7.1

Category Descriptions Used In Terrestrial Habitat Assessment

HABITAT CATEGORY	DESCRIPTION
Arable	All fields under arable cultivation, which are ploughed at least once a year.
Boundary	Hedgerows and Dry stone walls which may provide potential refugia or habitat corridors.
Garden	Rural and Suburban garden habitats and allotments
Long grassland	Grassland which is long for all or part of the year and has a litter component. This includes hay meadow and unimproved rank pastures.
Short grassland	Grassland that is short all year and has no significant litter component. This includes amenity grassland and improved heavily grazed pastures.
Urban/ Industrial	All large areas with tarmac or building complexes are included in this category as are factories and quarries.
Water	All open water is within this category including the study pond.
Woodland and Scrub	This describes any habitat where trees are the dominant component. Scrub is also included in this category.

7.2.5 Statistical Analyses

Since the habitats closer to the pond must be crossed by migrating adults and by emerging metamorphs they probably will influence the survival of a greater percentage of the population compared with more distant habitats. The habitat data was split into 2 sections for analysis: habitats further than 500m radius from the pond (>500m) and those within a 500m radius of the breeding site. Habitat variables were arcsine transformed to remove the unit-sum constraint of percentage variables. An outlier test was conducted on the transformed variables and any statistical outliers were removed before regression.

The population indices were logged and then regressed with the transformed habitat variables using male and female population indices as separate dependent variables.

These stepwise multiple linear regressions were carried out separately on habitat variables <500m and those >500m radius from the pond. A modified Bonferroni correction (Simes 1986) was applied to the results to correct for multiple testing.

An important factor in a recent model of the persistence of amphibian populations was initial population size (Halley *et al* 1996). If habitats surrounding a breeding site are not capable of maintaining an adequate population size, the population may go extinct. Halley *et al* (1996) calculated that persistence of a population had a probability of >95% if the average carrying capacity of the breeding site was greater than 30 adult females. This was termed the minimum viable population size (MVP). This paper was chosen as the basis for further calculations as it gives an MVP figure for *B. bufo* based on data from a population within the current study area (N.W. Leicestershire). Equations from the aforementioned multiple linear regressions were used to calculate the minimum area of habitats able to sustain an MVP of 30 females. Only regressions which were significant for both years were used in the calculations.

7.3 RESULTS

7.3.1 Population Indices

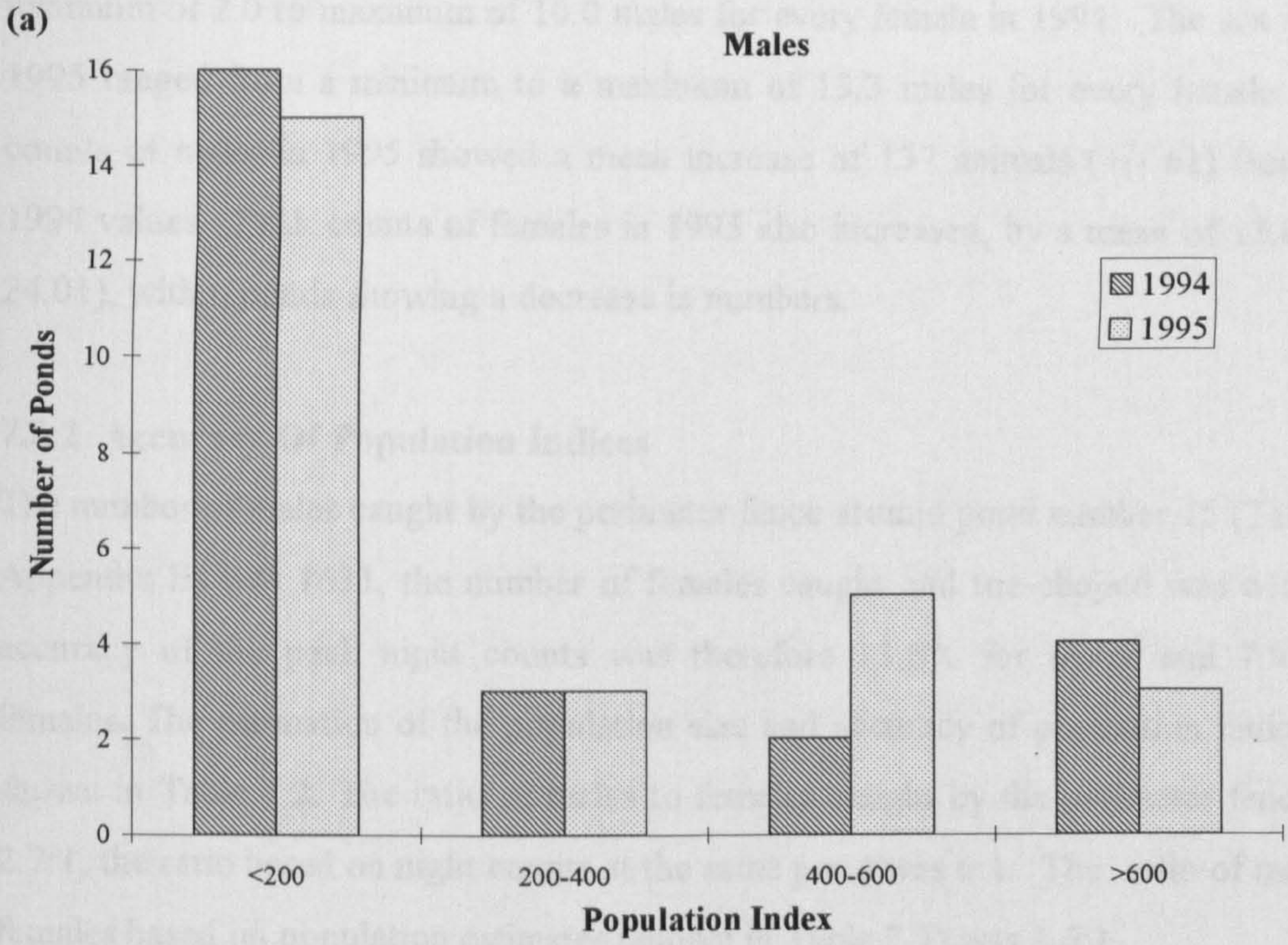
Only 26 of the 32 sites counted were used for analysis as recent land developments resulted in destruction or degradation of six of the chosen sites. The grid references, uncorrected population indices and a brief site description of all sites analysed are shown in Appendix E (Table E2).

The percentage of *B. bufo* on the surface of the water in the 20 clear water sites was 68.0 (+/- 8.02) in 1994 and 52.9% (+/- 7.72) in 1995. The 6 turbid ponds used in the analysis were therefore corrected by multiplying population indices by 1.47 in 1994 and 1.89 in 1995. The arcsine transformed percentage area of terrestrial habitats are given in Appendix E (Tables E3 a and b).

Figures 7.1 a and b give the frequency of pond indices for male and female *B. bufo* in both 1994 and 1995. Numbers of females seen were always smaller than numbers of males.

Figure 7.1

Frequency Distribution Of Population Indices



Population indices are the maximum or peak values of direct head counts of *B. bufo* at breeding sites. Data are corrected for water turbidity (see text for details)

The mean sex ratio based on peak number of males divided by peak number of females was 5.4 +/- 0.97 in 1994 and 8.7 +/- 2.03 in 1995. The sex ratio ranged from a minimum of 2.0 to maximum of 10.0 males for every female in 1994. The sex ratio in 1995 ranged from a minimum to a maximum of 13.3 males for every female. Peak counts of males in 1995 showed a mean increase of 137 animals (+/- 61) from their 1994 values. Peak counts of females in 1995 also increased, by a mean of 13.05 (+/- 24.01), with 6 ponds showing a decrease in numbers.

7.3.2 Accuracy Of Population Indices

The number of males caught by the perimeter fence around pond number 15 (Table E2 Appendix E) was 1631, the number of females caught and toe-clipped was 610. The accuracy of the peak night counts was therefore 13.8% for males and 7.9% for females. The estimation of the population size and accuracy of population indices are shown in Table 7.2. The ratio of males to females caught by the perimeter fence was 2.7:1, the ratio based on night counts at the same pond was 6:1. The ratio of males to females based on population estimates (shown in Table 7.2) was 3.5:1.

Table 7.2

Estimation Of Population Size And Accuracy OF Population Indices

	MALES	FEMALES
Number of toe-clipped Animals	1631	610
Percentage of animals caught in the water with a toe clip	66.7%	86.1%
N_{est} = estimated number at the breeding site	$1631 = 66.7\ \% N_{est}$	$610 = 86.1\% N_{est}$
Rearrange equation	$N_{est} = \frac{1631x100}{66.7}$	$N_{est} = \frac{610x100}{86.1}$
Number of *B. bufo* estimated at the breeding site	2445	708
Peak Population Index (Peak of night counts).	338	56
Accuracy of peak count	$13.8\ \% = \frac{338}{2445} \times 100$	$7.9\% = \frac{56}{708} \times 100$

The number of male and female *B. bufo* caught by the perimeter fence (day catches) and the number counted by the following night count are shown in Figures 7.2 a and b. For both sexes night counts and day catches showed a sharp and well defined peak on the 2nd and 3rd of April respectively. Female day catches showed a second peak on the 7th of April that was not reflected in the night count values. The first toe-clipped female to leave the pond was trapped on the 2nd of April and the first toe-clipped male to leave the pond was caught on the 4th April. Dates of peak migration from the pond were the 4th of April for females and 6th of April for males.

In Figure 7.2 the patterns of night counts follow those of the pitfall trap catches during the previous day. This suggests that the sum of the nightly counts would give a more accurate indicator of the population size than peak counts. The sum of all males seen on all night counts was 1607 which is 70% of the male breeding population size. The total number of females seen was 164 which was 24% of the female breeding population size

7.3.3 Habitat Categories And Population Indices

The mean percentage cover of each habitat variable and the standard error of the means are given in Figure 7.3. Arable and Short grassland had the largest mean areas both within 500m radius and further from the pond. All P values shown below are the Bonferroni corrected values.

(i) Habitats Further Than 500m Radius From The Breeding Site

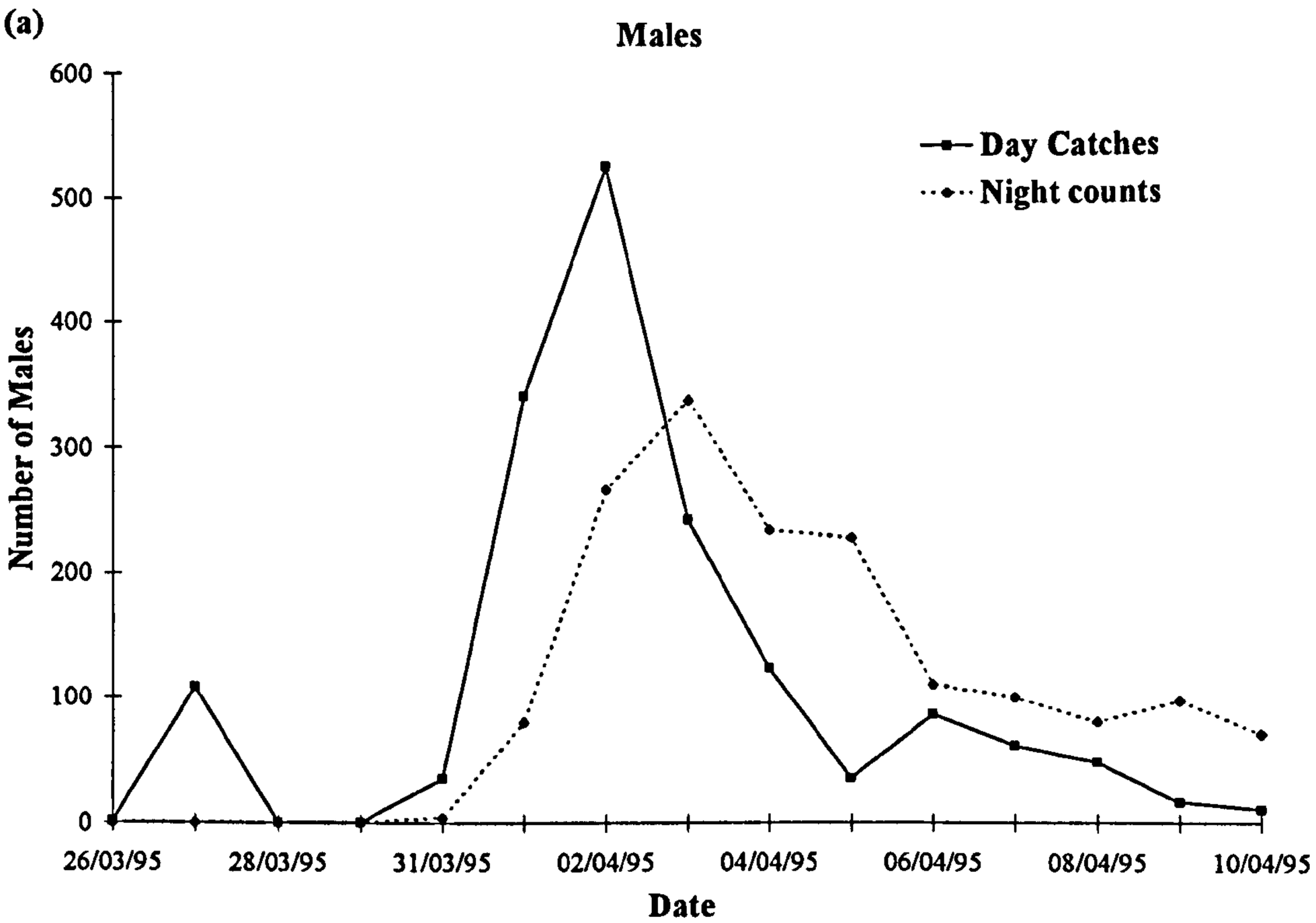
No habitat variables correlated significantly with the population indices of either male or female *B. bufo* in either 1994 or 1995.

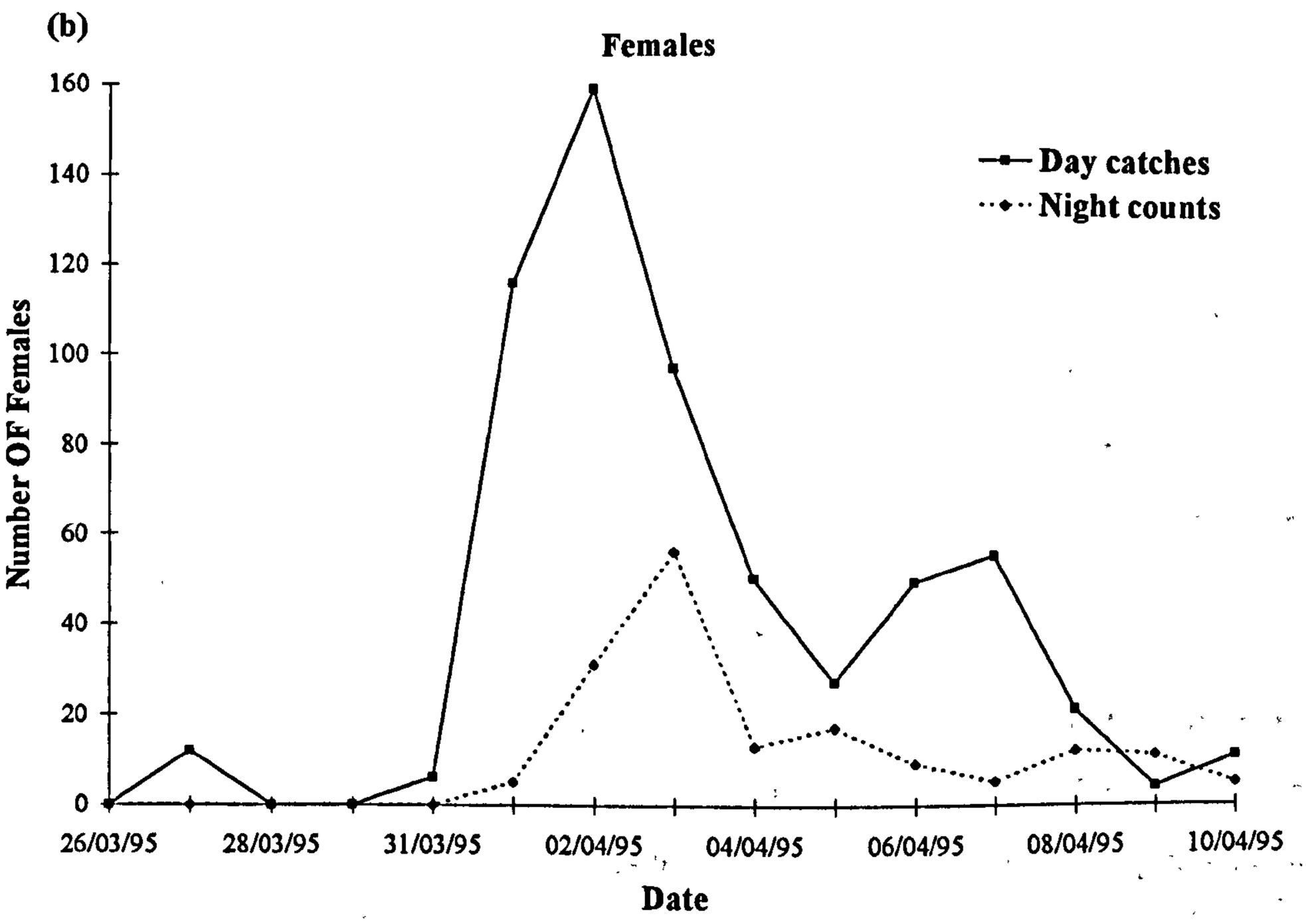
(ii) Habitats Less Than 500m Radius From The Breeding Site

In both 1994 and 1995 the population indices of males were positively correlated with the area of long grassland (R^2=0.48, $F_{1,23}$=21.42,P<0.01; R^2=0.47, $F_{1,23}$=20.30,P<0.01 respectively). The population of female *B. bufo* in 1994 and 1995 were also positively correlated with the area of long grassland (R^2=0.30, $F_{1,23}$=8.51,P<0.05; R^2=0.57, $F_{1,23}$=26.80,P<0.01 respectively).

Figure 7.2

Accuracy Of Night Counts Of *B. bufo*

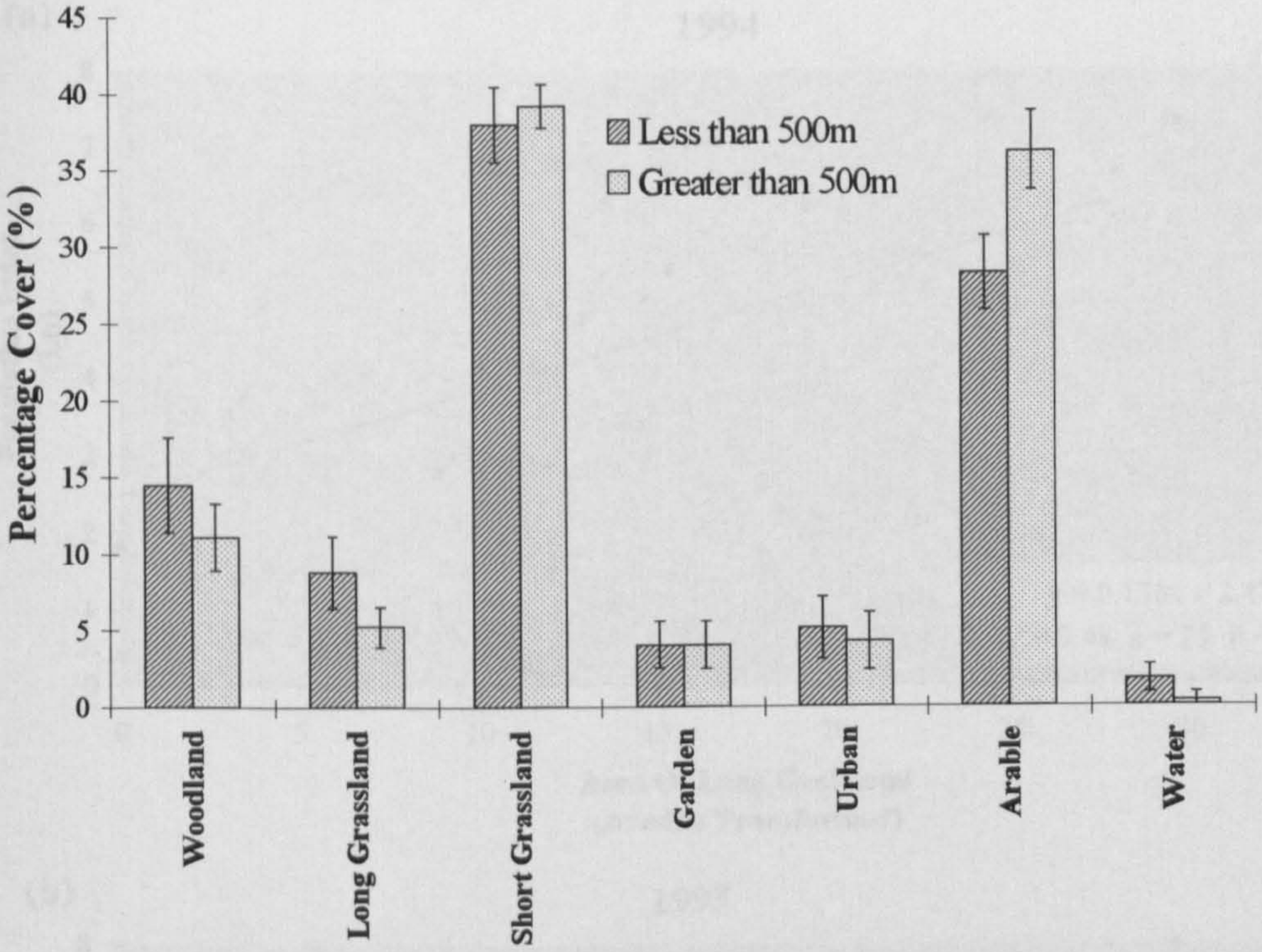




Day catches were the number of *B. bufo* caught entering the pond by the perimeter fence traps. These *B. bufo* were then placed into the water and allowed to mix with the breeding population. The night count values were the numbers of *B. bufo* seen by a single circuit torch count of the same pond.

Figure 7.3

Areas Of Terrestrial Habitat Categories Within 1000m Of The 26 Breeding Ponds Sampled

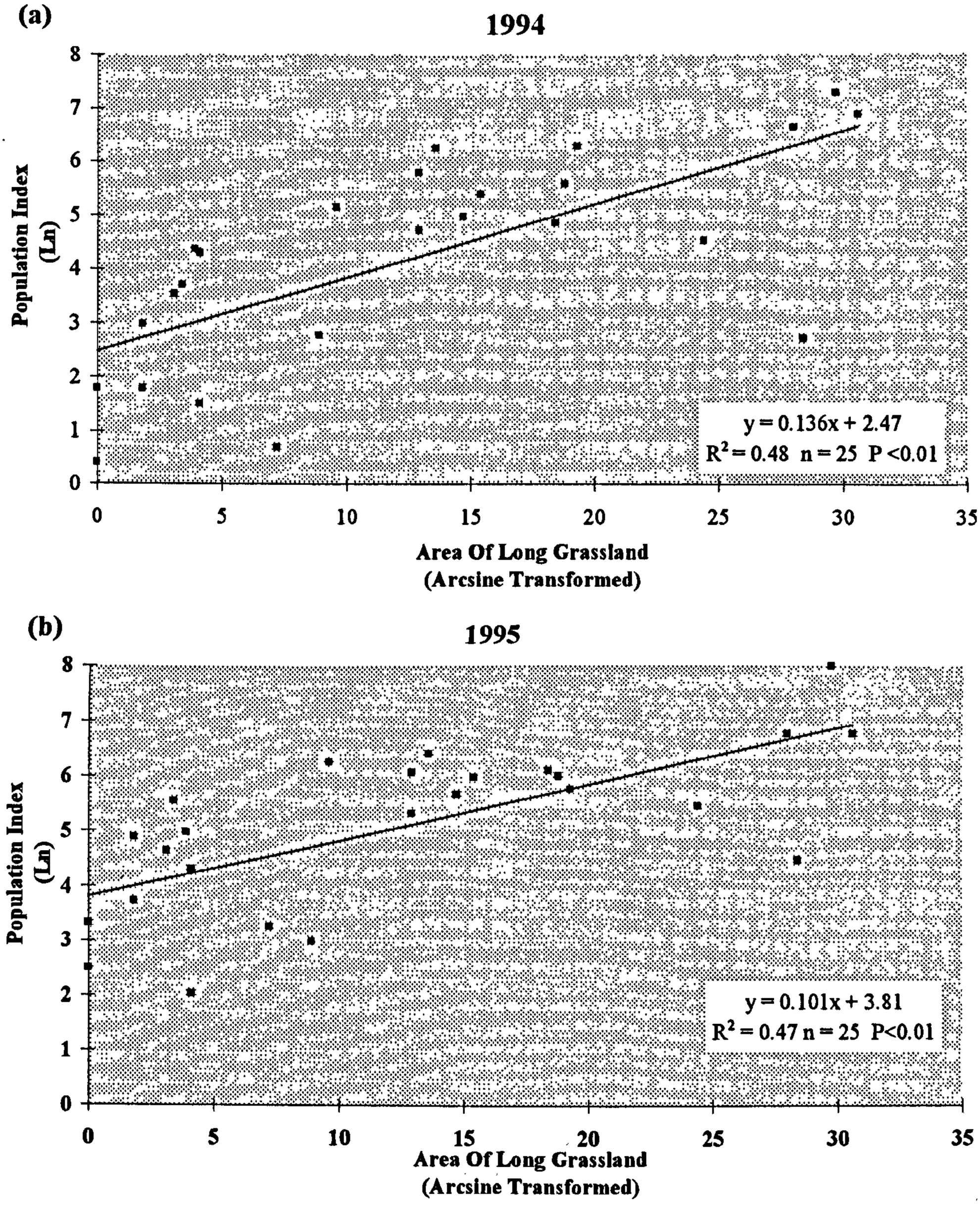


The regression equations and coefficients of correlation for interactions between population indices and area of long grassland are shown in Figures 7.4 and 7.5. The area of water was positively correlated to the 1994 female population indices (R^2=0.34, $F_{1,24}$=10.31,P<0.05) and the area of garden was negatively correlated to female population size in the same year (R^2=0.22, $F_{1,24}$=5.98,P<0.05). These remaining significant correlation coefficients and regression equations are given in Figures 7.6 and 7.7. The population indices of both sexes, from both years did not correlate with any other habitat variable significantly (P>0.05).

Rough grassland was the only habitat variable which correlated with population size of both males and females in both years. The area of long grassland which would sustain an MVP of 30 females was 11.38 hectares based on the 1994 regression equation and 6.20 hectares based on the 1995 regression equation. The calculation of these data are shown in Table 7.3.

Figure 7.4

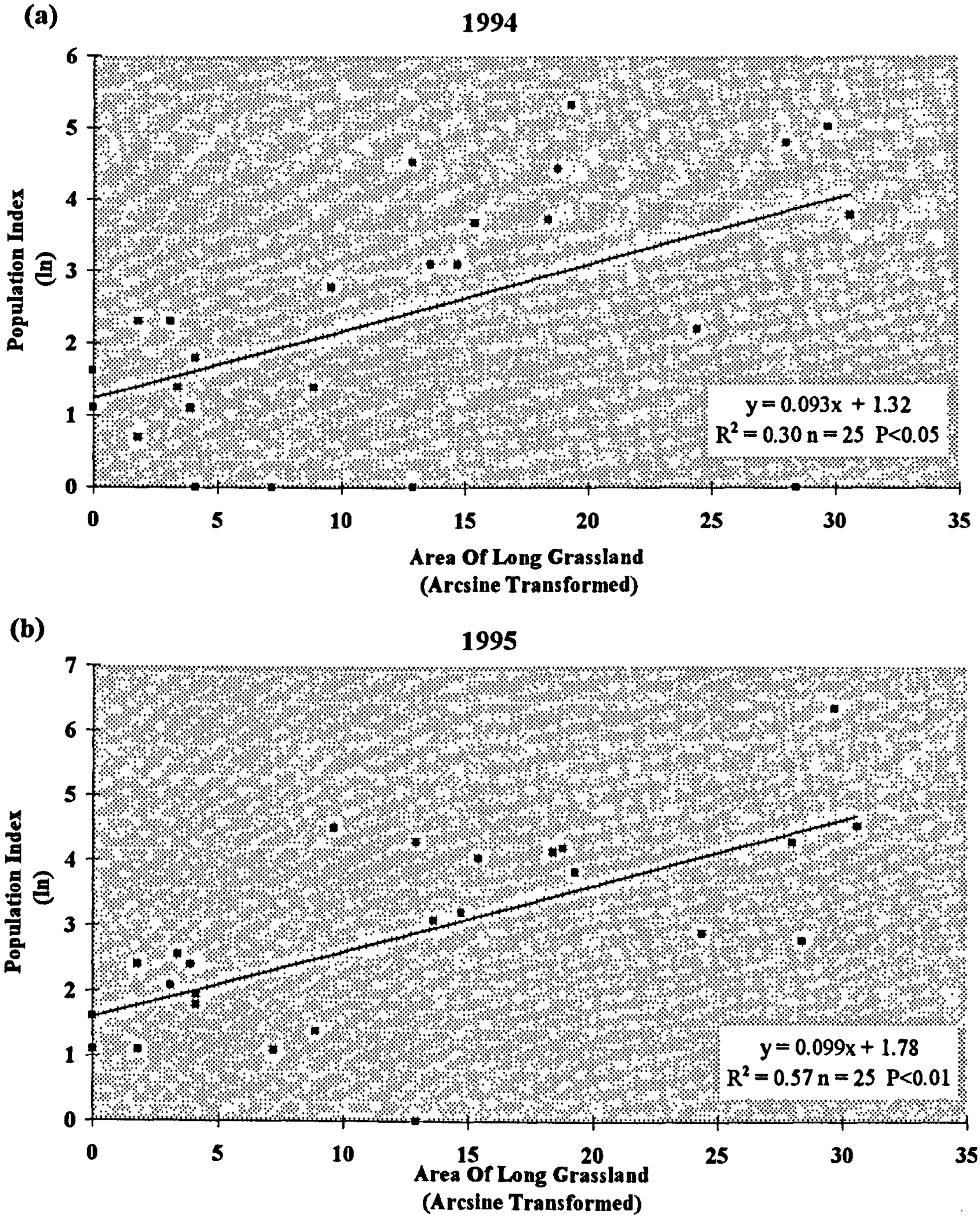
Population Indices Of Male *B. bufo* And Area Of Long Grassland Within 500m Radius Of The Breeding Site



The number of sites where the B. bufo population was counted = 26. There was one statistical outlier in regressions of population size and area of long grassland. Therefore n = 25.

Figure 7.5

Population Indices Of Female *B. bufo* And Area Of Long Grassland Within A 500m Radius Of The Breeding Site



The number of sites where the B. bufo population was counted = 26. There was one statistical outlier in regressions of population size and area of long grassland. Therefore n = 25.

Figure 7.6

Population Indices Of Female *B. bufo* In 1994 And Area Of Water Within A 500m Radius Of The Breeding Site

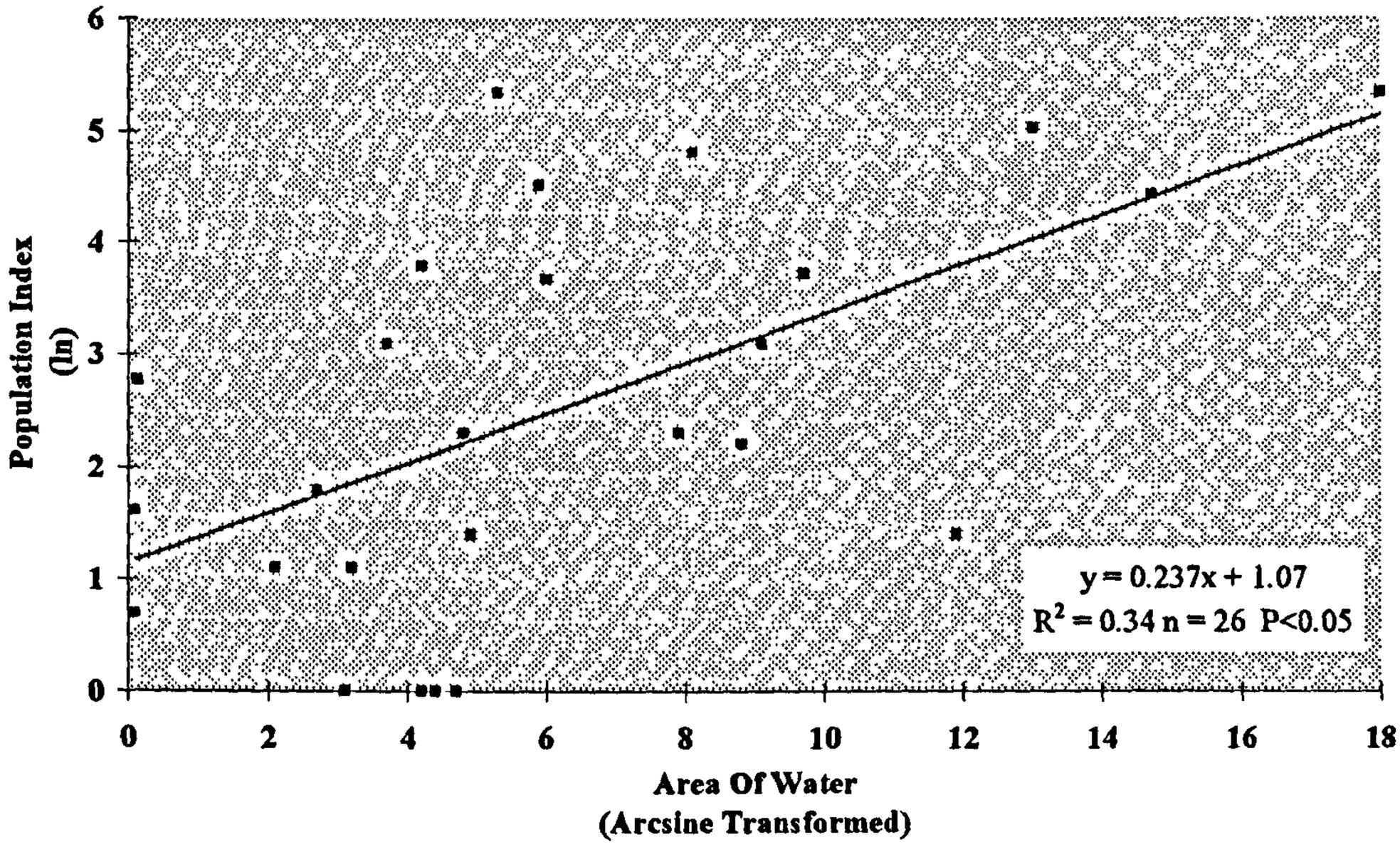
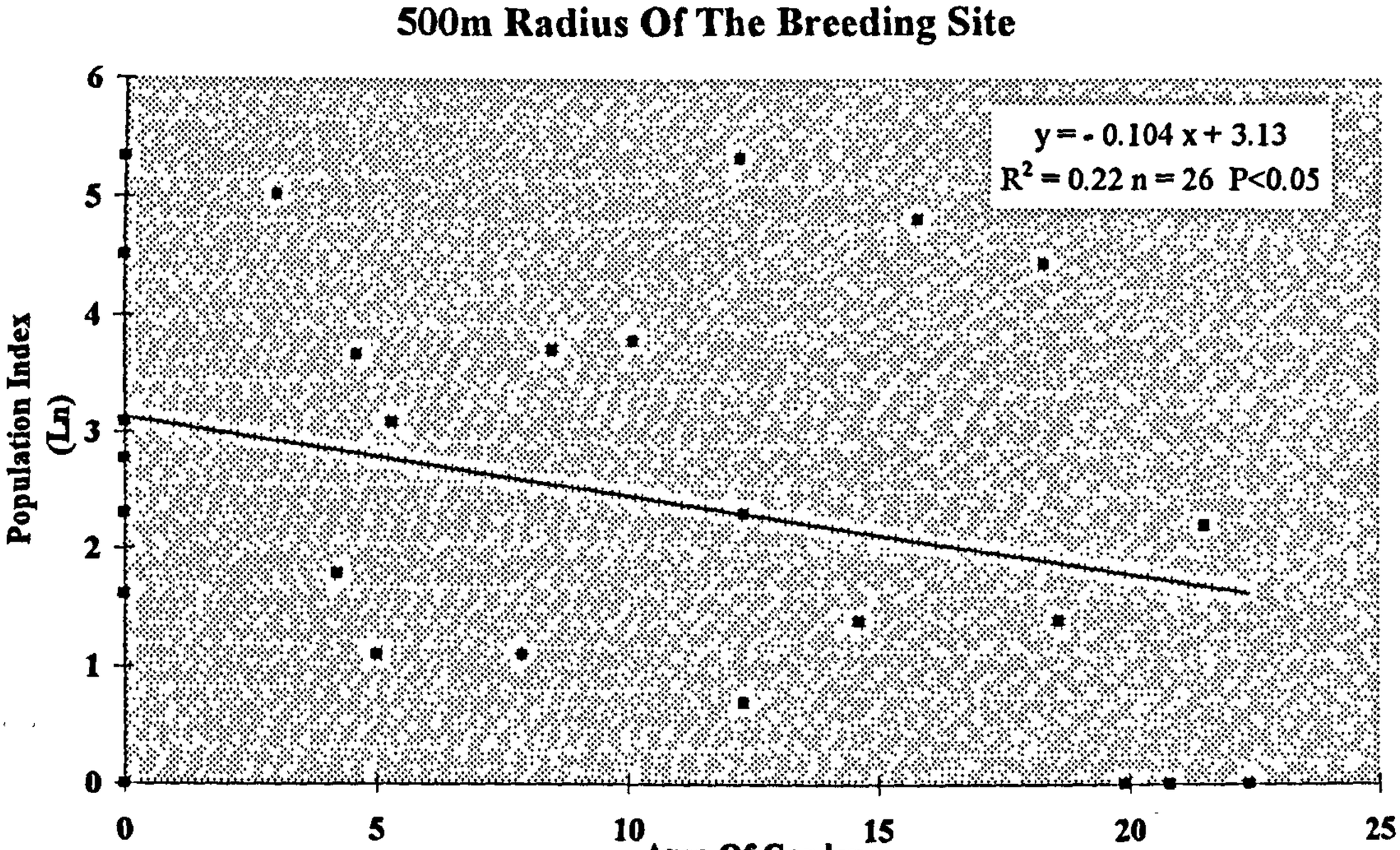


Figure 7.7

Population Indices Of Female *B. bufo* In 1994 And Area Of Garden Within A 500m Radius Of The Breeding Site



The number of sites where the B. bufo population was counted = 26. There were no statistical outliers in the above regressions therefore n = 25.

Table 7.3

Calculation Of Area Of Long Grassland Which Would Sustain An MVP Of 30 Female *B. bufo*

Regression equation	**1994** $y = 0.093x + 1.32$	**1995** $y = 0.099x + 1.78$
x = arcsine percentage area of long grassland in a 500m radius of the breeding pond. **y = ln female population index**		
Insert y = ln30	**x = 22.4**	**x = 16.38**
Back transformation	**14.49%**	**7.95%**
Total area used in analysis was a 500m radius from pond = 785,398m^2		
Conversion of percentage to unit area.	**11.38 Hectares**	**6.24 Hectares**

7.4 DISCUSSION

7.4.1 Population Distributions And Sex Ratios

The frequency distribution of peak counts for males and females are similar to those given by Cooke (1975). In the Cooke study the largest number of ponds had less than ten *B. bufo*. In this study the largest number of ponds were in the 10-100 category for females and the 100 to 999 category for males (Figure 7.1). The difference in distribution in the numbers of ponds in each population size category is possibly a reflection of the relative quality of recording in the two studies. The Cooke measurements were based on questionnaire survey results of amateur recorders, with uncertain and inconsistent methodology of population estimation often from only a single site visit.

The mean male to female ratios observed by night counting were 5.4 in 1994 and 8.7 in 1995. Male to female ratios observed ranged from between 2.0 and 10.0 males for every female in 1994 and from between 2.7 and 13.3 males for every female in 1995. Data from the current study suggests that a higher percentage of male *B. bufo* were seen by night counts compared with the percentage of females seen. This sex

differential in the percentage numbers seen was observed by Moore (1954). Night counts provided sex ratios of between 2.7 and 5.7 males to every female (Moore 1954). Data from migrating *B. bufo* at the same site placed the sex ratio at 2 males to every female. The sex ratio observed by night counts in the perimeter fenced pond in this study was 6.0 males to every female. The population estimates from catch and mark release recapture data in the current study revealed a sex ratio of 3.5 males to every female, lower than the 6.0 to 1 ratio obtained by night counts. Reading and Clarke (1983) studied a single population with a sex ratio of 2.73 males for every female and demonstrated that observed sex ratio is very variable. The sex ratios observed in the pond on any single night during the breeding season ranged from 2.5 to 12.7 (Reading and Clarke 1983).

The dichotomy between the percentages of females and males observed by night counts can be explained by the different breeding behaviours of the two sexes. Females come to the pond to spawn and leave or enter refugia immediately post spawning (Hoglund and Robertson 1987). Males are able to mate with more than one female and so some males remain for the entire breeding season (Davies and Halliday 1977, Gittins *et al* 1980). Males therefore arrive earlier and remain longer at the breeding site compared with females (Frazer 1966, Wisniewski *et al* 1980, Gittins *et al* 1980, Reading and Clarke 1983).

The ratio of 3.5 males to every female in the population estimates from the fenced pond is of the same order of magnitude as ratios observed by other workers using a variety of methods; 2.1 to 1 (Hoglund and Robertson 1987) 3.0 to 1 (Gittins *et al* 1980), 4.4 to 1 (Wisniewski *et al* 1980) and 4.9 to 1 Frazer (1966). Female *B. bufo* are less numerous due to the high energetic cost of mating which delays sexual maturation of the females by 1-2 years (Hemelaar 1983, Gittins *et al* 1980). Mortality in the years before sexual maturation results in the skewed sex ratios at breeding (Hemelaar 1983, Gittins *et al* 1980). Sex ratio varied from site to site in the current study which would be expected if there were differential sex based mortality at different sites. The number and types of predator did vary between sites (chapter 6)

however there is no data on sex based mortality at different sites from the current study.

7.4.2 Accuracy Of Population Indices

13.8% of males and 7.9% of female *B. bufo* present during the breeding season were counted in the peak night counts. Cooke (1975) multiplied the adult count by two to estimate of total population size. He gives no empirical evidence for this and the current data do not support it. The sum of all the night counts of males from the 31st March to the 10th April was 70% of the numbers actually present and gave an estimate closer to the actual population size than peak night count value. The sum night count value was influenced by the number of nights each site was visited, with the number of counts increasing for each visit. Further work is needed to determine how the percentage of the population seen by night counts varies between years and between sites. The accuracy of summed counts and peak counts need to be compared at a range of sites to determine the most suitable method for monitoring the population size of *B. bufo*.

Low percentage accuracy of peak night counts does not prohibit their use in comparative studies of *B. bufo* populations. If the percentage of the population observed was constant between ponds then night counts can be used for comparative studies. It was not possible to obtain this information, therefore the data from the population indices must be treated with caution.

This investigation was observational rather than experimental and therefore does not allow the inference of causality among the variables studied. The results must be interpreted with caution as correlations may be coincidental and many other factors, not least the quality of the aquatic habitat, will have influenced the population size. Added to this the percentage accuracy of the population indices may vary from site to site. The following discussion of the correlations between habitat variables and population indices must be interpreted with these caveats in mind.

7.4.3 Habitat Correlates Of Population Indices

Short grassland and arable were the largest percentage habitats in this study. This area of Leicestershire is known for dairy farming associated with large areas of improved pasture. Large areas of cereal crops are also grown in this region.

The area of three habitat categories closer than 500m from the breeding sites were correlated with *B. bufo* population counts. There were no significant correlation between habitat areas further than 500m from the breeding site and *B. bufo* population counts. This was expected as more of the population will be influenced by habitats immediately surrounding the pond (Oldham and Swan 1991). Adults must cross habitats close to the pond to breed and to their summer home ranges. Emerging metamorphs must cross the habitats immediately surrounding the pond to reach their first over-wintering sites (Oldham and Swan 1991).

In chapter 3 the habitat preference of *B. bufo* given a choice of habitats were discussed. The most preferred habitat for male and female *B. bufo* was the long grassland. Long grassland was also the only variable which correlated with the population indices of both male and female *B. bufo* in both years for habitats close to the breeding site.

Area of water within a 500m radius of the breeding pond was positively correlated with the number of female *B. bufo*. This variable is mainly the breeding pond area and the correlation can be explained by the higher carrying capacity of larger aquatic habitats (e.g. Cooke 1975).

Area of garden was negatively correlated to the population size of female *B. bufo* in 1994. Cooke (1975) found an association between small *B. bufo* populations and garden ponds. Beebee (1979) and Mathias (1975) found *B. bufo* were the least common of the amphibians studied, in surveys of amphibian presence in garden ponds. The ponds near large areas of housing would be subject to more disturbance than those further into the countryside. It is possible that the disturbance from human activity

combined with the unsuitability of most well kept suburban gardens limit the populations in these areas.

Sex specific differences in habitat correlations could be explained by sex specific differences in habitat preference and concomitant survival. Sex specific differences may also arise from differences in the accuracy of night counts for males and females discussed in section 7.4.1.

To make absolute statements about the influence of terrestrial habitat on the population size of *B. bufo* from these data is impossible. Two main points limit the conclusions that can safely be drawn from this study: the problems involved in the measurement of population size, and the time scale of the study (2 years). Studies of natural fluctuations in population size must by definition be observational, and there is no other method of studying a large number of common *B. bufo* populations without an impractical number of man hours. The correlation of population size and long grassland was however consistent between years and sexes despite large fluctuations in the population indices from 1994 to 1995. The most preferred habitat of radio-tracked *B. bufo* was rough grassland which would fit into the long grassland category in Table 7.1 offering strong support for the importance of this habitat category.

Increasing the area of long grassland to greater than 6.2 hectares may increase the carrying capacity of the terrestrial habitat if this is the limiting factor. Management of terrestrial habitats to include large areas (>6.2-11.4 hectares) of rough grassland (or other habitats with a litter component) should have a carrying capacity large enough to sustain an MVP if the aquatic habitat is also carefully managed. If the aquatic habitat is the limiting factor then altering the availability of terrestrial habitats will not alter the population size.

7.5 CONCLUSIONS

- 13.8% of male and 7.9% of female *B. bufo* present at a breeding site were recorded by the peak of night counts. The sum of night counts was a more accurate index of *B. bufo* population size.

- Data on the comparative accuracy of night counts between ponds and between years are required.

- The sex ratio of male to female *B. bufo* caught by the perimeter fencing at one pond was 2.7:1. The sex ratio observed by night counts at the same pond was 6 to 1. The sex ratio of night counts at 26 breeding sites ranged from a maximum 13.27 to a minimum of 2.67 in 1995 and from a maximum of 10.00 to a minimum of 2 males for every female in 1994 .

- Only habitats within a 500m radius of the breeding site were correlated with *B. bufo* population indices.

- Female population indices in 1994 were significantly positively correlated with the area of water and negatively correlated with the area of garden less than 500m radius from the breeding site.

- Area of long grassland within 500m radius of the pond was significantly correlated with the population indices of both male and female *B. bufo* in both 1994 and 1995.

- The area of long grassland which would sustain an minimum viable population (MVP) of 30 females was 11.4 hectares based on the 1994 regression equation and 6.2 hectares based on the 1995 regression equation.

8: DISCUSSION AND CONCLUSIONS

The niche a species occupies is defined by a series of dimensions along which resources are partitioned. Macrohabitat, microhabitat, food type, food size, diel and seasonal time are the main ecological factors that can be partitioned between and sometimes within species (Schoener 1974, Norberg 1977, Begon *et al* 1990). These dimensions can be influenced by macro and microclimates and dimensions may interact. Preference for a specific habitat is an example of habitat partitioning, which may be influenced by microclimate. Prey selection is food partitioning and may also be influenced by microclimate. This thesis determined the terrestrial dimensions of the niche of *B. bufo*. Figure 8.1 summarises the interactions between these dimensions and their potential influence on habitat selection and population size.

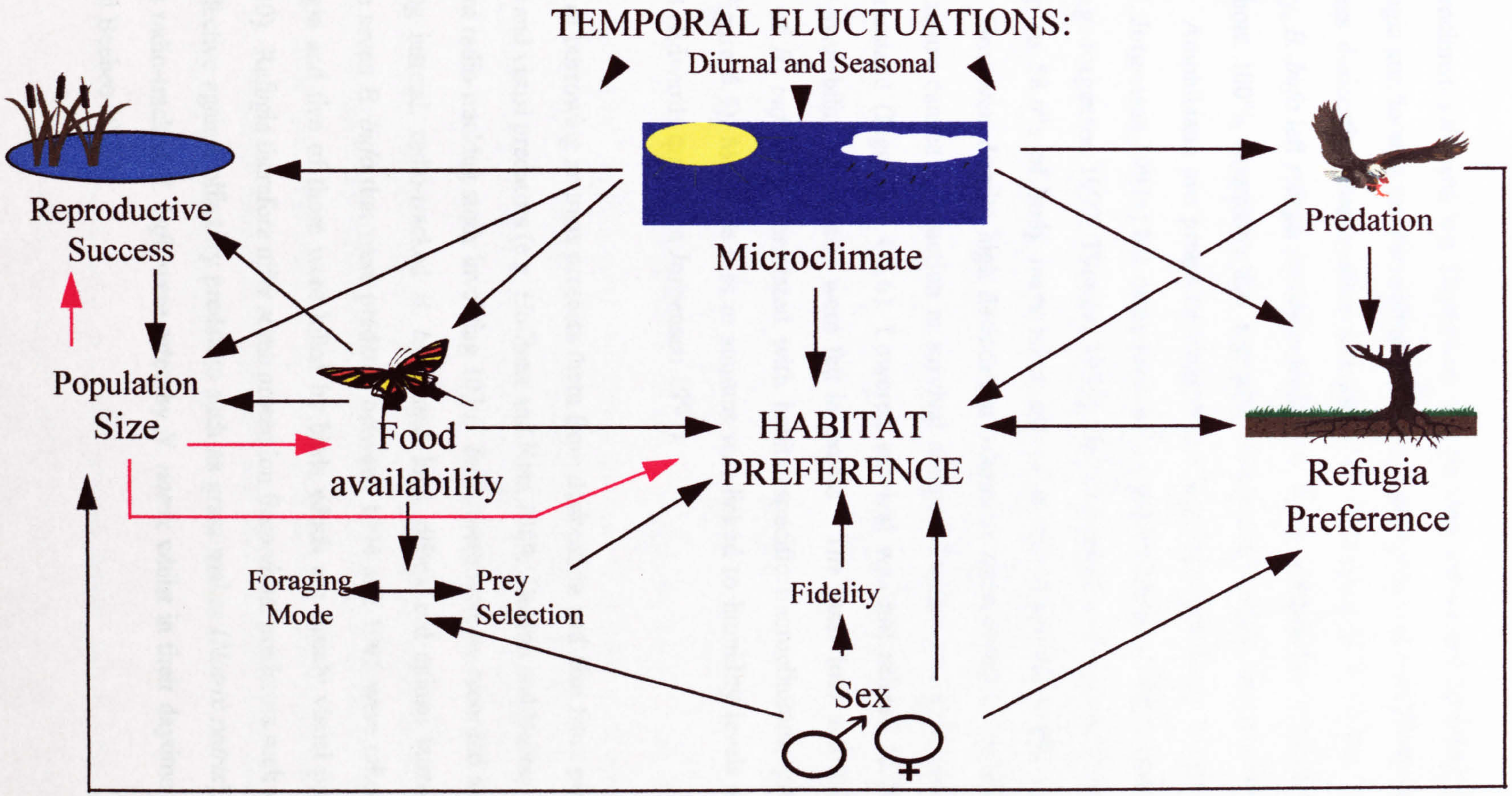
Habitat is generally the first and most important dimension partitioned by both amphibians and reptiles (Toft 1985). In the current study habitat is a selected resource as radio-tracked *B. bufo* demonstrated a significant preference for rough grassland and woodland over arable and improved pasture habitats. The importance of habitat partitioning by British anurans has been demonstrated by Denton and Beebee (1994). They showed the two British bufonids segregated mainly by terrestrial habitat, as food niche overlap was high. This terrestrial niche partitioning has important conservation implications for the endangered natterjack toad. Changes in terrestrial habitat have caused a breakdown in allopatry between these two *Bufo* species and this has lead to local extinctions of *B. calamita* (Banks *et al* 1994, Denton *et al* 1997).

8.1 HABITAT PREFERENCE AND MICROCLIMATE

Radio-tracked *B. bufo* demonstrated two distinct forms of behaviour: active; when above ground and alert, showing an ability to orientate towards prey, and passive; in refugia. Refugia were either below ground, sometimes in animal burrows, or above ground under leaf litter or in rotting vegetation (chapter 3). Rough grassland and woodland were preferred by active animals and were used almost exclusively for refugia by passive animals (Figure 3.5). Improved pasture and arable lacked a litter component and had no woody perennials which form root hollows and this limited the availability of refugia in these habitats.

Figure 8.1

Schematic Representation of The Correlates of Habitat Preference



Arrows show the direction of influence of a variable. Temporal fluctuations influence all other factors. Red arrows are possible pathways for density dependent feedback. For detail see text.

Microclimates of woodland and rough grassland were significantly cooler and more humid than the arable and improved pasture habitats (Figures 6.7-6.10). The influence of microclimate on the habitat preference is represented schematically in Figure 8.1.

Refugia microclimates showed low fluctuations of both temperature and humidity (Figure 6.11). Refugia ameliorated the microclimates of rough grassland and woodland which in turn were less desiccating than pasture and arable habitats (Figures 6.7-6.10). In the current study, *B. bufo* left refugia mostly at night and during precipitation when humidity was at or about 100%, suggesting that a primary function of refugia was prevention of desiccation. Amphibians are prone to evaporative moisture loss (e.g. Thorson and Svihla 1943, Jorgensen 1991a) but many terrestrial amphibians have a high desiccation tolerance (e.g. Jorgensen 1997, Thorson 1955). In the current study *B. bufo* survived losses of up to 58.6% of body mass when placed in non-preferred habitat-specific enclosures. However, despite high desiccation tolerance, microclimatic conditions in improved pasture caused a reduction in survival compared with survival in woodland and long grassland (Figures 6.4-6.6). Lowered survival was not related to habitat-linked prey availability as animals were fed in excess. The mass loss and therefore desiccation of *B. bufo* was correlated with habitat-specific microclimates, primarily humidity (Figure 6.5). Moisture loss in anurans was linked to humidity levels as long ago as 1824 (Edwards quoted in Jorgensen 1997).

The refugia of burrowing anurans protects them from desiccation and also from predation by olfactory and visual predators (e.g. Hoffman and Katz 1989, Denton and Beebee 1993). In the current radio-tracking study involving 103 *B. bufo*, there were no recorded instances of burrowing instead, radio-tracked *B. bufo* used logs, debris and animal burrows for refugia. The seven *B. bufo* that were predated between 1994 and 1995 were taken when out of refugia and five of these were killed by birds which are mainly visual predators (Section 6.10). Refugia therefore offer some protection from visual predators such as birds but are ineffective against olfactory predators such as grass snakes (*Natrix natrix*). Three out of eight radio-tracked *B. bufo* were eaten by *N. natrix* whilst in their daytime refugia (Denton and Beebee 1993).

Anurans have a considerable spatial awareness (e.g. Collett 1982) and many species show fidelity to the same breeding site from year to year (e.g. Reading *et al* 1991). *Bufo bufo* have been shown to return to the same home range after the spring migration (e.g. Haapanen 1974, Sinsch 1988). Habitat preference may therefore be influenced by genetic or learned fidelity to specific habitats. The evidence from this study suggests that fidelity is not a primary determinant of habitat preference. Radio-tracked *B. bufo* were from two discrete populations with little or no interbreeding and yet there was no significant difference in the habitat preferences, either active or refugia, between these two populations (Section 3.5.3).

8.2 FEEDING AND FORAGING MODE

Amelioration of climatic extremes by the preferred habitats probably increased the potential opportunities of *B. bufo* to feed. Movement into arable and improved pasture habitats from woodland and rough grassland increased desiccation and predation costs but provided increased food availability. Arable and improved pasture had a significantly higher percentage abundance and a significantly greater dry mass of Coleoptera than either woodland or rough grassland (Figures 4.8, 4.9 and 4.11) Arable also had significantly larger invertebrates than woodland or rough grassland (Figures 4.5 and 4.6). Invertebrate taxa length and calorific value are usually related (Schoener 1961) therefore arable had higher calorific prey availability than woodland or rough grassland. The increased feeding benefits of these habitats were reflected in the diet of *B. bufo* from outside the radio-tracking area. *B. bufo* feeding in arable and pasture habitats had significantly higher dry mass in their diets than *B. bufo* from woodland and rough grassland (Figure 5.3).

B. bufo moved into arable and improved pasture when climatic conditions minimised cost and maximised benefits. Daily maximum temperatures when *B. bufo* moved into arable or pasture were lower than days when *B. bufo* remained active within preferred habitats (Figure 6.12b). Minimum temperatures, minimum and mean humidity were also higher when arable or pasture were used (Figure 6.12a).

Activity of invertebrates and therefore toad and prey encounter rate is influenced by microclimate (e.g. Briggs 1960, Greenslade 1961, Cornish 1992). Total invertebrate

invertebrate numbers trapped was positively correlated with maximum humidity and minimum temperature (Figures 4.14 and 4.15). High humidity and high minimum temperatures are found on overcast or rainy nights, which are associated with increased anuran activity (e.g. Sinsch 1988). These conditions prompt anura to leave refugia (Duellman 1995). Before *B. bufo* moved into arable and improved pasture habitats, climatic conditions reduced the risk of desiccation and increased invertebrate activity and therefore prey encounter rate.

This cost/benefit scenario can explain the differences in active habitat use between 1994 and 1995. Refugia habitat preference did not differ with sex or between 1994 and 1995 (Table 3.4). The order of habitat preference of females in both years and males in 1995 was identical (Table 3.5). The percentage use of arable and improved pasture habitats varied with year and with sex mostly due to the behaviour of the males in 1994. Arable and improved pasture were used significantly more by males in 1994 than in 1995 (Figure 3.6). 1994 was significantly cooler and wetter than 1995 (Figures 6.7-6.10). The dry conditions in 1995 reduced activity of the invertebrates producing a lower total dry mass and a reduced coleopteran dry mass (Figure 5.3). In 1995 the desiccation costs were higher and the prey benefits of arable and pasture were lower than in 1994. This leaves open the question of why females were not similarly affected by the climate difference between 1994 and 1995.

Sex specific differences in use of habitats may have resulted from differences in desiccation tolerance or in feeding behaviour/prey preference. Radio-tracked females were larger than the males and therefore had a lower surface area to volume ratio. This may be thought to reduce the potential for desiccation as evaporative water loss can be linked to surface area and as size increases the area increases proportionally less than the volume. However Thorson (1955) found tolerance to desiccation within a species decreased with size (length and hydrated mass) because the percentage of body water decreased with size. Therefore females may be more prone to desiccation and consequently less able to utilise the open habitats than the males in 1994.

Female *B. bufo* had greater total dry mass in their diets than males due to their larger size. Females also ate more larger taxa and a greater dry mass of Coleoptera than males. Females may gain more benefit from a single visit to Coleoptera-rich feeding areas such as arable than do the smaller males. Thus females may require fewer feeding occasions than males to obtain the equivalent or greater calorific/nutritive value. This will only be possible if there are sex specific differences in the efficiency of feeding. Sex specific differences in diet linked to habitat and microclimate were found in the tusked frog *Adelotus brevis* (Katsikaros and Shine 1997). Males had more taxa in diets and ate a higher percentage of small vertebrates than females. Differences were not related to the larger gape width of males, but were linked to habitat preference and therefore food availability. Females spent most of their time feeding in drier microhabitats. Katsikaros and Shine (1997) did not measure food availability in different habitats.

Arthropodivores have traditionally been categorised as either sit-and-wait strategists, where prey are passively located, or as widely-foraging, where time and energy are spent searching for food (Schoener 1961). Foraging mode and degree of prey selectivity are linked in anurans (Toft 1981) and in lizards (Anderson and Karasov 1981). Sit-and-wait strategists ambush their prey and tend to eat prey in proportion to availability, whereas widely foraging species tend to show a degree of specialisation. In studies of *B. bufo* (where prey availability was not recorded) *B. bufo* have been described as unselective (Gittins 1987). As observed by Simon and Toft (1991) diet is often presumed to be opportunistic when there is no measure of prey availability in the environment.

In the current study, *B. bufo* ate a wide range of prey taxa. Prey availability was also considered, which allowed electivity indices and preference to be evaluated. *B. bufo* demonstrated a prey size preference and possibly a prey taxon preference. Prey preference was probably related to the detectability of taxa, as big prey were fast moving and within the right size range for vision. Selection for prey size rather than taxon is common amongst salamanders and lizards (Toft, 1985).

Table 8.1 summarises the features common to sit-and-wait and widely foraging lizards (Huey and Pianka 1981) and anurans (Toft 1981). Sit-and-wait predators commonly eat mobile prey, have low daily energy expenditure, are cryptic, have limited learning ability and are not poisonous. Observations from the current study (also listed in Table 8.1) show *B. bufo* is intermediate between the two foraging types. *B. bufo* has the majority of features of a typical ambush strategist but with higher mobility, greater learning ability, toxic skin secretions and sometimes eats "clumped prey" e.g. ants. Toft (1981,1985) found some anurans with intermediate morphology and behaviour. These intermediate anurans were generalists and fit the behaviour, morphology and prey selection patterns of *B. bufo* observed in the current study. The intermediate morphology and behaviour are possibly due to the complex annual cycle of *B. bufo* described in Figure 1.1. During spring, and to a lesser extent in autumn, *B. bufo* migrate over long distances which increases the potential for *B. bufo* to be preyed upon. Toxic skin secretions and high mobility are necessary to reduce predation and complete this stage of their annual cycle in the shortest possible time.

Though *B. bufo* is primarily a sit-and-wait strategist it moves between areas of high prey availability when environmental conditions are favourable. The diet of *B. bufo* in the current study changed with habitat type reflecting habitat specific changes in prey availability. Since *B. bufo* are able to learn and move around they are able to adapt to different prey availability under different microclimates. *B. bufo* feeding on ants in nests is an example of a patchy resource which must be located by search behaviour. If there are large numbers of sedentary prey with patchy distributions, active foragers will have a higher encounter rate than sit-and-wait strategists (Toft 1980).

Movement into areas of high resource level when environmental conditions are appropriate could be described as facultative sit-and-wait behaviour. A similar facultative sit-and-wait behaviour was found in the Leptodactylid frog *Ceratophrys cornuta* (Duellman and Lizana 1994). Plasticity of foraging mode with changes in food availability has been demonstrated in lizards by Huey and Pianka (1981) and in anurans by Toft (1980). The flexibility is limited by morphology and physiology as well as sensory mode and learning ability.

Table 8.1

General Correlates Of Foraging Mode In Litter-Dwelling Anurans

	Sit-and-Wait[1]	*B. bufo*[2]	Widely Foraging[1]
Prey type	Large, mobile	Preference for large prey - small prey also taken	Small, slow-moving prey
Number of Prey	Low	Intermediate	High
Anti-predator Defence	Cryptic coloration	Cryptic coloration, Skin toxins	Skin toxins/aposematic coloration
Morphology	Stocky, wide-mouthed	Stocky, wide-mouthed	Slim, narrow-mouthed
Sensory Mode	Primarily visual	Primarily visual	Visual or Chemoreceptory
Learning ability	Limited	Enhanced learning and memory.	Enhanced learning and memory.

1 = data are adapted from Toft (1981) and Huey and Pianka (1981). 2= data are taken from the current study.

8.3 POPULATION SIZE AND HABITAT PREFERENCE

Aquatic and terrestrial habitat loss have been cited as contributing to the documented declines of most British amphibians (Cooke 1972, Beebee 1977b, 1979, Cooke and Ferguson 1974, Cooke and Scorgie 1983, Swan and Oldham 1989, Grayson *et al* 1991). In the current study population size was linked to habitat availability around 26 *B. bufo* breeding sites. Area of long grassland within a 500m radius of the pond was significantly correlated with the population indices of both male and female *B. bufo* in both 1994 and 1995 (figures 7.5 and 7.6). Long grassland was the closest land use category to rough grassland, the preferred habitat in the radio-tracking enclosure, emphasising the importance of habitats with a litter component.

Studies have linked declines of *B. bufo* and *T. cristatus* to habitat loss as a result of changes in land use practices (e.g. Cooke and Ferguson 1974, Hilton-Brown and Oldham 1991). Land use practices (habitat categories) were related to population size in the current study (Chapter 7) and land use, therefore has direct conservation implications. An important factor in a recent model of the persistence of amphibian populations was initial population size (Halley *et al* 1996). Halley *et al* (1996)

calculated that persistence of a population had a probability of >95% if the average carrying capacity of the breeding site was greater than 30 adult females. Based on data from the population and habitat surveys from chapter 7, the area of long grassland which would sustain a minimum viable population size (MVP) of 30 females was between 6.2 and 11.4 hectares.

The carrying capacity of the terrestrial habitat will depend upon the availability of suitable refugia and food. The data from the current study suggests that a mosaic of semi-natural and arable land which allows for easy movement between high food availability sites and low desiccation risk refugia, would probably provide a high carrying capacity for *B. bufo.* The mosaic nature of habitats influences prey availability; the presence of semi-natural and cultivated land in small mosaics increases the abundance of Coleoptera (Duelli *et al* 1990). Semi natural habitats, such as rough grassland, are important for over-wintering of *Carabidae*, as they reduce temperature fluctuations (Descender *et al* 1981)

There is strong evidence that terrestrial habitat has a significant influence on aspects of population structure and dynamics in amphibians. The body size and sexual maturation rates of *R. temporaria* can vary in different terrestrial habitats (Augert and Joly 1993). The species richness of amphibian communities alters with habitat type and degree of fragmentation (Mann *et al* 1991). Altering terrestrial habitats by clear-felling reduced terrestrial amphibian populations by up to 70% in the coastal forests of Canada (Dupuis *et al* 1995). Size of *B. bufo* at breeding and fecundity in females, may be influenced by the juvenile terrestrial environment (e.g. Swan 1986). Terrestrial habitat choice and availability are therefore important in the population dynamics and structure of *B. bufo.* The current study provides more evidence of the importance of habitat preference. Habitat around a breeding site influenced food availability (chapter 5), survival (chapter 6) and population size (chapter 7).

Though habitat loss is still the major cause of amphibian declines in Britain (Swan and Oldham 1989), concern is growing over the effects of pollution. For example, anthropogenic acidification of surface waters can be a problem for British amphibians.

Tadpoles of *R. temporaria* at low pH showed reduction in growth rates and increased percentage deformity (e.g. Cummins 1989). Beebee *et al* (1990) gave evidence that anthropogenic acidification of breeding sites had contributed to the reduction in the distribution of the Natterjack toad (*B. calamita*) during the 20th Century.

Global declines in anurans have been linked to altered weather patterns (e.g. Bury *et al* 1980, Crump *et al* 1992, Fellers and Drost 1993, Pounds and Crump 1994). Altered amphibian breeding patterns in the United Kingdom have also been linked to climate change (e.g. Beebee 1995). Climate change may result in prolonged stay in refugia by some anurans. For example *B. marinus* was active during wet nights but during protracted periods of dry weather there was no activity for up to several days (Dobkin *et al* 1989). The current study suggests that *B. bufo* would have fewer opportunities to feed and fewer foraging opportunities in open habitats if weather conditions became drier. Climatic conditions were correlated to the body condition (and therefore fecundity) of *B. bufo* by Reading and Clarke (1995). They studied a population of *B. bufo* at a breeding site in Dorset and found that *B. bufo* were more likely to enter hibernation in poor condition following a hot dry summer than after a wet summer.

Differences in the climatic conditions between 1994 and 1995 affected habitat use, movement and feeding of *B. bufo*. Reduced opportunities to feed would eventually impact on population size via reduced condition and fecundity. Production of both male and female gametes is dependent on the nutritional status of *B. bufo* as they enter hibernation (Jorgensen *et al* 1978, Guha *et al* 1980). Reduction in the body condition of *B. bufo* and concomitant reductions in fecundity have been linked to climate change (Reading and Clarke 1995). The importance of climate change on amphibian population declines remains contentious as not all populations are declining (e.g. Duellman 1995) and some workers have linked epidemic disease to declines in pristine environments (e.g. Laurance 1996). A full discussion of the importance of climate change on the global amphibian declines is outside the remits of this thesis, however further study of the effects of climate change on temperate zone anurans is warranted.

8.4 CONCLUSIONS

Radio-tracking with ingested transmitters can be used to provide information on the behaviour of *B. bufo* however transmitter to toad mass ratio must be <10%. When transmitters with a mean mass of 2.5g were ingested, *B. bufo* with a mass below 25g, showed reduced feeding and loss of body mass. Radio-tracked *B. bufo* in both the active phase and when in refugia (passive phase) preferred woodland and rough grassland over arable and pasture. Refugia provided significant amelioration of microclimatic extremes and served to reduce desiccation stress and predation of passive phase *B. bufo*.

Microclimates of the preferred habitats also reduced desiccation stress of *B. bufo* compared to arable and improved pasture. The preferred habitats within the radio-tracking enclosure were more humid and ameliorated temperature extremes observed in the arable and pasture. *B. bufo* placed in non-preferred habitat enclosures lost mass and had reduced survival. The loss of mass was significantly correlated to minimum humidity.

The non-preferred habitats were better potential feeding grounds than woodland and rough grassland. Arable and pasture provided a higher dry mass of invertebrates in general, and Coleoptera in particular. Coleoptera were the largest constituent of the *B. bufo* diet in terms of dry mass. When diet was compared to invertebrate availability Coleoptera had the highest positive electivity in all three years of the study and had the highest dry mass of all taxa in the diet of *B. bufo*. The diet of *B. bufo* differed between habitats reflecting differences in the availability of prey in these habitats. There were higher dry masses of Coleoptera in the stomachs of *B. bufo* from arable and pasture than those from woodland and rough grassland habitats.

The habitat preference of *B. bufo* differed between years and between sexes due to the increased use of non-preferred habitats by male *B. bufo* in 1994. The difference in habitat use between the sexes did not seem to be related to sexual dimorphism in feeding behaviour. Males and females had similar percentage abundance of invertebrate taxa in their diets. The main dietary difference between male and female *B.*

bufo was the higher dry mass of invertebrates in female stomachs which, was correlated with their larger size.

Terrestrial habitat influences the population dynamics of *B. bufo*. Population size of males and females were significantly positively correlated to the area of long grassland (the most preferred habitat category) in both survey years. To sustain a population of *B. bufo* above the minimum viable population size, habitats within a 500m radius of the breeding pond should be managed to contain a minimum of between 6.2 and 11.4 hectares of long grassland and/or woodland.

B. bufo showed plasticity of diet in different habitats reflecting habitat-specific differences in prey availability. In the mosaic of habitats within an agricultural landscape, *B. bufo* were able to utilise areas of high prey availability, but potentially desiccating microclimates, when macro-environmental conditions were favourable.

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APPENDIX A

A Survey Of The Flora In The Radio-tracking Enclosure Habitats

A1 Methods

Forty co-ordinate pairs chosen from four sets of random numbers were used to locate the position of forty $1m^2$ quadrats. Ten quadrats were placed in each of the four main habitats; woodland, rough grassland, arable and improved pasture. Vascular plants found within each quadrat were identified to species level where possible using field guides (Rose 1981, Aichele 1987 and Sinker 1975). Species of forbs were not identified but percentage ground cover of forbs was noted when greater than 10%. The percentage ground cover of each species was estimated in categories following the Braun-Blanquet scale shown in Table A1 (after Kent and Coker 1995). The modal Braun-Blanquet value from the 10 quadrats for each species is given in Table A2. If the modal value was 0 but the plant was recorded in one or more quadrats a value of + was recorded in Table A2. In the woodland the species of trees were identified and all the trees in the woodland were counted. The numbers of each tree species is shown in A2 in place of the Braun-Blanquet value.

Table A1

Vegetation Abundance Categories

BRAUN-BLANQUET VALUE	COVER SCALES
+	Less than 1% cover
1	1 - 5 % cover
2	6 - 25 % cover
3	26 - 50% cover
4	51 - 75% cover
5	76 - 100% cover

A2 RESULTS

Table A2

Species Within The Radio-tracking Enclosure Habitats

HABITAT	SPECIES*[1]		ABUNDANCE CATEGORY*[2]
Arable	Cultivated varieties of:	*Allium cepa*	2 - 3
		Brassica oleracea Var *capitata*	2 - 3
		Brassica napus Var *olfeira*	4 - 5
		Lactuca sativa	3
		Pisum sativum	3 - 4
		Solanum tuberosum	4 - 5
	Ruderal weeds	*Agropyron repens*	+ - 1
		Chenopodium Album	+ - 1
		Lamium album	+ - 1
		Other *Gramineae*	+ - 1
		Rumex crispus	+ - 1
		Rumex obtusifolius	1
		Taraxacum officinale aggregate	+ - 1
		Urtica dioica	1
Improved Pasture		*Capsella bursa-pastoris*	3
		Lolium perenne	4 - 5
		Polygonum aviculare	3 - 4
		Rumex crispus	+
		Rumex obtusifolius	1
		Taraxacum officinale aggregate	+ - 1
		Veronica filiformis	+
Woodland	Trees	*Malus species*	1*[2]
		Quercus robur	1*[2]
		Sambucus nigra	1*[2]
		Ulmus procera	2*[2]
		Fraxinus excelsior	5*[2]
	Ground Layer	*Anemone nemerosa*	+
		Aegopodium podagraria	+
		Hedra Helix	3 - 4
		Galeopsis tetrahit	1
		Lamium album	1 - 2
		Lamium purpureum	+ - 1
		Polygonyum convolvulus	+
		Ribes nigrum	2 - 3
		Rumex obtusifolius	3 - 4
		Ranunculus ficaria	+
		Ranunculus repens	+
		Urtica dioica	2 -3
		Vicia sepium	+ - 1
		Viola riviniana	+

*[1] Species are listed in alphabetical order of genus in each of the four habitats. Species names are as listed in Rose 1981. *[2] The number of trees in the woodland is recorded and not the abundance category.

Table A2 (Cont..)

Species Within The Radio-tracking Enclosure Habitats

HABITAT	SPECIES*[1]	ABUNDANCE CATEGORY*[2]
Rough Grassland	*Agropyron repens*	+
	Anthriscus sylvestris	1
	Arrhenatherum elatius	2 - 3
	Carduus acanthoides	+
	Chelidonium majus	+
	Chenopodium album	+ - 1
	Cirsium eriophorum	+
	Cirsium vulgare	+
	Festuca rubra	1
	Galeopsis tetrahit	1
	Galium cruciata	+
	Geranium robertianum	+
	Hedera helix	+
	Heracleum sphondylium	+
	Lamium album	1 - 2
	Lolium perenne	1 - 2
	Poa annua	1
	Poa trivialis	1
	Prunella vulgaris	+
	Ranunculus repens	+ - 1
	Reseda lutea	+
	Rumex crispus	1
	Rumex obtusifolius	2 - 3
	Senecio vulgaris	+
	Silene dioica	+
	Taraxacum officinale aggregate	+
	Trisetum flavescens	1
	Urtica dioica	2
	Veronica filiformis	+
	Vicia lathyroides	+
	Vicia sepium	+

APPENDIX B

Table B1

Tukey Test Summary - Percentage Abundance of Invertebrate Taxa Caught In Suction Traps

		TAXA											
YEAR		1993				1994				1995			
HABITAT		W	R	A	P	W	R	A	P	W	R	A	P
1993	W		Ac, Pu	Ac, Co	Ac, Co, Dt	Ac, Op	Ac, Pu	Ac, Co	Ac				Op
	R			Co, Di, Pu	Co, Di, Dt, Pu	Ac , Op	Ac, Di, Pu	Ac, Co	Ac, Di			Di	Di,Pu, Op
	A				Dt	Ac, Co, Dt, Pu, Op	Co, Dt, Pu	Dt	Co, Pu	Co	Co, Di	Co, Dt	Co, Dt, Op
	P					Co, Op	Co, Pu		Dt	Ac, Co	Co, Di	Co	Co, Op
1994	W						Pu, Op	Co, Op	Dt, Op, Pu	Ac, Op	Ac, Op	Pu, Op	Ac, Pu, Op
	R							Co, Pu	Dt	Ac, Pu	Ac, Di, Pu	Ac, Pu	Ac, Op
	A								Co, Dt, Pu	Ac, Co	Ac, Co	Ac, Co	Ac, Co, Op
	P									Ac, Dt	Ac, Di	Ac, Dt, Pu	Ac, Dt, Pu, Op
1995	W											Co, Dt	Dt, Op
	R											Di, Dt, Co	Dt, Pu, Op
	A												Di, Op
	P												

Letters are taxa whose cell means are significantly different

Ac = Acari
Co Coleoptera
Di = Diplopoda
Dt = Diptera
Op = Opilone
Pu = Pulmonata

W = woodland, P = pasture, Rough grassland, A = arable.

APPENDIX C

Note all tables with times to digestion are after Whittaker (1995). All other tables are from observations of 128 stomach contents for details see chapter 4.

Table C1

TOAD STOMACH CONTENTS - DIGESTION RATES

(a) ARANAE (SPIDERS)

STAGE	IDENTIFICATION POINT	GENERAL DESCRIPTION
1	Whole animal	Firm, colour retained, legs present and intact. Shape maintains integrity.
2	Body softens and outline loses definition (fuzzy)	Body Firm but all or some of the legs will be lost up to middle or lowest joints. The outline of larger spiders becomes fuzzy.
3	Body begins to break up and starts to soften	Body can separate in large spiders. Rear of abdomen is soft and flaccid and may become transparent. Body and head are soft but not transparent.
4	Body in pieces, some pieces missing. Soft and very digested.	Legs often become detached at this stage, abdomen often missing, whole thorax and head are pale flaccid and becoming transparent. Body parts like an empty sack
5	Ghost or/and pieces of leg.	Only pieces of leg left. A ghost of a shape mainly held together by water tension.

(b) COLEOPTERAN LARVAE (AND DIPTERAN LARVAE)

STAGE	IDENTIFICATION POINT	GENERAL DESCRIPTION
1	Whole animal	Firm, has clearly defined edges (not fuzzy). No decomposition, is opaque and head is obviously different from body in Coleoptera.
2	Edges fuzzy	Slight decomposition of the body wall cause a lack of definition and the edges of the larvae become fuzzy. Quite firm and still opaque
3	Becomes flaccid and soft.	As decomposition continues, the insides of the larvae digest and the body is soft, flattened and flaccid. There is still some diameter to the body and much of the body is opaque though end will become transparent
4	Becomes transparent	Is pale flaccid and may loose the rear of the larvae. Head will still be intact and recognisable at this stage.
5	Ghost/sack	The inside of the larvae is completely digested and the larvae is now just a sack or transparent tube.

(c) DERMAPTERA

STAGE	IDENTIFICATION POINT	GENERAL DESCRIPTION
1	Whole animal	The animal is intact with all parts joined and no flesh visible below the chitin segments of the abdomen.
2	Segments soften	The chitin on the abdominal segments softens and flexes. This allows the flesh to show. The abdomen is soft and flat but still intact.
3	Abdomen begins to break up.	The abdominal segments begin to break away from the rest of the animal.
4	Abdomen, head and thorax in separate pieces	The individual pieces of the animal are still recognisable, but they are separate or break apart on contact. The chitinous segments have softened and are digesting.
5	Only pieces left, usually just head	Only a few pieces are left undigested usually just the head and pincers of the tail.

(d) DIPLOPODA (AND CHILOPODA)

STAGE	IDENTIFICATION POINT	GENERAL DESCRIPTION
1	Whole animal	The segments are firm, coloured and intact.
2	The animal, breaks up into large segments.	The animal, breaks up into large segments but is still firm and opaque. Colour may dull.
3	Segments soften and number of segments increases	Segments break apart on touch and number of separated segments increases. The separate pieces are still large and the whole individual can be identified.
4	Splits into separate segments and segments soften.	The segments are almost all separate, and have become spongy. The colour has paled and some legs have detached.
5	Just pieces of segment	As digestion continues the segments separate completely and become mashed and paler. The individual animal is impossible to distinguish. The legs become detached from the segments and are digested.

(e) DIPTERA

STAGE	IDENTIFICATION POINT	GENERAL DESCRIPTION
1	Whole animal	No decomposition, edges clearly defined and body firm and opaque.
2	Body softens	Wings of smaller Dipterans detach. Whole body still visible. In larger Dipterans the abdomen starts to become pale.
3	Soft, pale, flaccid	The body parts will usually separate on contact. The abdomen is either missing or pale and flaccid. the edges of the body are fuzzy.
4	Ghost	Often head or abdomen missing. Body is pale and decomposing. Wings may be still attached to thorax of larger Dipterans.
5	Just wings	Only wings and bits of leg left.

(f) FORMICIDAE

STAGE	IDENTIFICATION POINT	GENERAL DESCRIPTION
1	Whole animal	Animal still whole and in natural shape.
2	Animal curls into ball	Animal is still whole but has been curled into a ball by the processes of digestion. The abdomen becomes paler or transparent.
3	Begins to break up	Some parts are still attached but heads and legs may detach. The abdomen is now very transparent and the chitin is softening.
4	In large pieces	Ants are broken up into constituent parts but legs are still whole.
5	In small pieces	Decomposition nearly complete. Only pieces of leg and pieces of head e.g jaw are left

(g) HOMOPTERA (APHIDS)

STAGE	IDENTIFICATION POINT	GENERAL DESCRIPTION
1	Whole animal	Firm and colour still obvious
2	Whole animal begins to digest	Slightly transparent, slightly paler, softer, less coherent than 1.
3	Some legs missing	Body goes fuzzy at edges and softens
4	Ghost	Becomes flaccid and only has outer layers of body wall. Becomes transparent
5	Ghost	Ghost just held together by water tension and will disintegrate if moved with forceps.

(h) ISOPODA (WOODLICE)

STAGE	IDENTIFICATION POINT	GENERAL DESCRIPTION	TIME
1	Animal is whole an segments uncracked	The animal is still whole and curled. The segments are uncracked and mostly undamaged	0 hours +
2	Cracks form in segments	The segments begin to crack and the armour begins to break. The flesh becomes visible and the segments are still rigid. The chitin joints are still intact.	7 hours +
3	Segments lose rigidity. Animal still mostly whole	The segments have mostly lost their rigidity and can flex and bend. The chitin joints begin to break down . The segments of the body are still mostly intact.	9 hours +
4	Joints break and legs and head begin to loosen	The chitin joints break and the segments are now held together by the digestive tract. Legs and head come off or loosen and segments begin to split up.	16 hours +
5	Segments split off and only pieces left	The segments split up and the head becomes very loose or detaches. The chitin becomes transparent and the legs become detached. Eventually becomes a grey formless mush.	19-24 Hours

(i) OLIGOCHETA (EARTHWORMS)

STAGE	IDENTIFICATION POINT	GENERAL DESCRIPTION	TIME
1	Body wall intact	The animal is still undigested. There is no splitting of skin.	0 hours +
2	Splitting appears on the body wall.	The worms split either horizontally or/ and vertically. Intestinal tract may be exposed by the split. The *body wall remains* joined.	2 hours +
3	Body begins to break into segments	The pieces which form between the vertical and horizontal splits, break into segments and may hang together by epidermal cells. The gut now begins to digest.	*4 hours +*
4	Segments are more obvious. The stomach almost totally digested	The segments become smaller and more finely divided and the worm is hardly intact at all. There is little remaining of the stomach and the body wall is in small pieces.	6 hours +
5	Mouth and wall are all that remain of worm	The mouth and wall are in small pieces and the worm has completely lost integrity.	8 hours - 9 hours

(j) PULMONATA (SLUGS)

STAGE	IDENTIFICATION POINT	GENERAL DESCRIPTION	TIME
1	Slug skin still intact	No splitting animal is still whole and the body wall is not distended	0 hours +
2	Wall distended but not split	Animal will still be whole but the body wall distended usually around gut area	3 hours +
3	Body wall splits	The intestines begin to force their way out of the split.	5 hours +
4	Body wall begins to digest and intestines also digest	The animal no longer maintains its' integrity. The body wall splits at several places. The intestines and body begin to digest	7 hours +
5	Mostly only digestive tract remains	The body has mostly been digested and most of the body wall has gone. Only digestive tract remains obvious.	9 hours +

Table C2

Dry Mass of Taxa In The Diet Of Male and Female *Bufo bufo*

	Woodland and Rough Grassland		Arable and Pasture		Unclassified	
	Male	Female	Male	Female	Male	Female
Acari	0.05 +/-0.04	0.81 +/-0.59	0.08 +/-0.06	0.00 +/-0.00	5.06 +/-4.76	0.29 +/-0.014
Coleoptera	123.5 +/-43.66	48.51 +/-13.64	152.1 +/-36.29	101.3 +/-47.91	120.4 +/-39.32	114.9 +/-38.9
Collembolla	0.12 +/-0.09	0.02 +/-0.00	0.00 +/-0.00	0.00 +/-0.00	0.00 +/-0.00	0.00 +/-0.00
Dermaptera	8.7 +/-4.55	0.00 +/-0.00	15.96 +/-9.74	10.61 +/-6.18	11.56 +/-3.89	18.01 +/-8.76
Diplopoda	6.59 +/-3.44	11.59 +/-4.70	15.73 +/-5.23	2.3 +/-2.30	4.4 +/-1.84	3.05 +/-1.94
Diptera	0.36 +/-0.14	0.37 +/-0.11	2.31 +/-1.13	0.26 +/-0.26	1.76 +/-0.91	5.45 +/-5.08
Hemiptera	3.95 +/-2.43	2.56 +/-1.37	1.05 +/-0.43	1.38 +/-1.32	20.29 +/-16.46	3.08 +/-1.06
Hymenoptera	8.21 +/-7.82	0.37 +/-0.23	0.9 +/-0.41	1.2 +/-0.97	11.96 +/-4.83	6.43 +/-2.14
Isopoda	29.58 +/-10.46	12.33 +/-5.23	2.03 +/-1.11	7.3 +/-4.47	8.96 +/-4.87	2.28 +-1.77
Larvae	74.38 +/-40.52	39.21 +/-34.58	62.16 +/-31.08	126.2 +/-61.00	79.6 +/-49.89	22.24 +/-20.11
Opilones	14.45 +/-7.04	3.55 +/-1.68	3.53 +/-1.31	15.13 +/-7.67	11.09 +/-5.31	5.33 +/-2.37
Other	22.34 +/-2.28	36.23 +/-20.22	14.96 +/-3.02	6.73 +/-3.45	21.24 +/-6.32	9.17 +/-2.50
Pulmonata	2.79 +/-2.28	5.69 +/-3.32	4.05 +/-3.77	1.01 +/-1.01	1.59 +/-0.72	1.25 +/-0.91

Table C3

Tukey Test Summary - Dry mass of taxa in Diet of *Bufo bufo*

		Female		Male	
Sex	Habitat	Woodland and rough grassland	Arable and pasture	Woodland and rough Grassland	Arable and pasture
Female	Woodland and rough grassland		Ac, Di, Op, Co, Dt	Di, Op, Co, Dt	Ac, Di, Co, Dt
	Arable and Pasture			Ac, Di, Op, Co, Dt	Di, Op, Co, Dt
Male	Woodland and rough grassland				Ac, Di, Op, Co, Dt
	Arable and Pasture				

Data are taxa whose mean dry mass are significantly different.
Ac = Acari Co= Coleoptera Di = Diplopoda Dt = Diptera Op = Opilones

Table C4

Tukey Test Summary - Percentage Abundance of Taxa In Diet of *Bufo bufo*

		Female		Male	
Sex	Habitat	Woodland and rough grassland	Arable and pasture	Woodland and rough Grassland	Arable and pasture
Female	Woodland and rough grassland		De, Is La	Pu	Di, He, Is, La
	Arable and Pasture			De, He, Is, La	
Male	Woodland and rough grassland				De, Pu, He, Is, La
	Arable and Pasture				

Data are Taxa whose mean percentage abundance are significantly different
Di = Diplopoda. De = Dermaptera. He = Hemiptera. Is = Isopoda. Pu - Pulmonata. La = Larvae

Table C5(a)

The Diel Electivity Indices Per Taxa

	Electivity Index D		
	1993	1994	1995
Acari	-0.31	-0.06	-0.17
Coleoptera	0.21	0.34	0.27
Collembola	-0.71	-0.51	-0.33
Dermaptera	-0.00	0.04	0.13
Diplopoda and Chilopoda	0.22	0.06	0.08
Diptera	-0.12	-0.00	-0.31
Hemiptera	-0.07	0.03	0.01
Hymenoptera total	0.13	0.14	0.26
Isopoda	0.10	0.14	0.17
Larvae	0.00	0.00	0.10
Opilones and Aranae	-0.07	-0.05	-0.05
Other	0.00	0.06	0.13
Pulmonata	-0.07	0.04	-0.06

Table C5(b)

The Nocturnal Electivity Indices Per Taxa

	Electivity Index D	
	1994	1995
Acari	-0.45	-0.65
Coleoptera	0.42	0.33
Collembola	-0.28	-0.47
Dermaptera	0.17	0.18
Diplopoda and Chilopoda	0.07	0.14
Diptera	0.04	-0.06
Hemiptera	0.06	0.00
Hymenoptera total	0.12	0.12
Isopoda	0.07	0.06
Larvae	0.00	0.07
Opilones and Aranae	-0.24	-0.10
Other	-0.06	-0.04
Pulmonata	0.03	-0.01

Shaded values are positive electivity indices of selected taxa. Other = all other taxa in diet /available in different habitats. Oligochaeta. are included in this category. Larvae = invertebrate larvae mainly coleopteran, dipteran and lepidopteran larvae. All values were calculated using the formulae shown in section 5.3.3. F_k in table 5.2 was calculated from combining suction trap data with diel pitfall trap data. F_k in table 5.3 was calculated from combining suction trap data with nocturnal pitfall trap data. For details see section 5.3.3.

APPENDIX D

Table D1

Mean Mass of *Bufo bufo* in Single Habitat Enclosures -1993

HABITAT	DAY						
	1	2	3	4	5	6	7
Rough Grassland	11.24 (+/-0.86)	12.50 (+/-0.96)	11.36 (+/-1.34)	12.55 (+/-1.11)	10.67 (+/-1.52)	13.15 (+/-1.35)	14.00 (+/-1.00)
Woodland	11.99 (+/-1.15)	11.26 (+/-1.37)	10.80 (+/-1.41)	10.17 (+/-2.00)	11.92 (+/-0.74)	14.57 (+/-0.96)	13.97 (+/-2.43)
Pasture	12.96 (+/-0.87)	12.34 (+/-0.80)	12.24 (+/-0.98)	11.85 (+/-1.25)	12.96 (+/-0.72)	12.03 (+/-1.00)	11.45 (+/-1.65)

Table D2

Mean Mass of *Bufo bufo* in Single Habitat Enclosures -May 1995

HABITAT	DAY						
	1	2	3	4	5	6	7
Rough Grassland	17.78 (+/-0.73)	17.23 (+/-0.69)	16.97 (+/-0.69)	16.89 (+/-0.70)	16.99 (+/-0.70)	17.23 (+/-0.71)	16.34 (+/-0.65)
Woodland	17.60 (+/-0.83)	17.06 (+/-0.84)	17.12 (+/-0.92)	17.03 (+/-0.99)	17.10 (+/-0.97)	17.38 (+/-0.95)	13.44 (+/-0.78)
Pasture	17.77 (+/-0.52)	15.80 (+/-0.51)	14.83 (+/-0.58)	14.25 (+/-0.63)	13.59 (+/-0.59)	13.44 (+/-0.78)	15.58 (+/-0.48)

Table D3

Mean Mass of *Bufo bufo* in Single Habitat Enclosures -June 1995

HABITAT	DAY						
	1	2	3	4	5	6	7
Rough Grassland	15.24 (+/-0.53)	15.40 (+/-0.49)	14.91 (+/-0.56)	14.19 (+/-0.41)	13.90 (+/-0.36)	13.94 (+/-0.43)	14.24 (+/-0.53)
Woodland	16.19 (+/-0.62)	15.57 (+/-0.58)	14.86 (+/-0.58)	14.19 (+/-0.65)	14.44 (+/-0.65)	13.79 (+/-0.61)	13.37 (+/-0.78)
Pasture	16.78 (+/-0.73)	14.71 (+/-0.57)	13.59 (+/-0.47)	11.99 (+/-0.52)	12.05 (+/-0.45)	12.33 (+/-0.44)	10.53 (+/-0.69)

Table D4

Temperature in Radio-tracking Enclosure - 1994

DATE	Pasture			Rough Grassland			Woodland			Arable		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
30.06.94	17.66	13.6	23.8	17.59	23.8	13.3	17.63	14.0	23.4	18.19	14.3	24.2
01.07.94	21.89	6.8	45.3	16.26	32.9	8.6	15.07	10.1	19.6	20.98	7.2	44.0
02.07.94	20.80	13.6	38.8	17.32	23.4	13.3	15.42	13.3	18.2	21.02	14.3	36.8
03.07.94	20.86	12.6	42.2	16.99	22.0	13.3	15.48	13.6	18.2	20.87	14.1	39.3
04.07.94	20.60	14.7	36.8	17.44	23.1	14.7	15.80	14.3	17.8	20.07	15.1	30.6
05.07.94	18.19	10.4	33.3	15.15	19.2	11.5	13.93	11.5	16.1	17.72	11.9	32.9
06.07.94	17.03	10.1	32.5	14.49	20.7	10.8	13.42	11.1	16.1	16.54	11.1	28.8
14.07.94	19.90	13.3	33.3	17.68	23.3	13.3	16.09	13.6	19.2	20.24	12.6	34.1
15.07.94	18.40	11.8	31.5	16.29	21.3	11.9	15.28	12.6	17.5	19.36	12.2	32.5
16.07.94	19.70	9.1	32.3	14.79	23.1	9.7	13.98	10.4	17.5	17.68	9.3	33.7
17.07.94	18.90	9.0	35.0	14.94	30.3	10.1	14.28	10.8	18.2	18.52	9.0	38.0
18.07.94	19.00	8.9	36.4	14.69	31.4	9.7	14.26	10.4	17.8	18.33	9.0	39.3
19.07.94	19.93	10.1	31.5	15.59	20.7	10.4	14.71	11.5	18.2	19.27	10.1	34.1
20.07.94	20.20	12.2	31.1	17.12	31.4	12.2	15.74	12.9	19.6	21.23	11.5	39.6
21.07.94	20.28	13.3	29.2	17.34	22.2	12.5	15.95	12.6	17.1	21.32	12.9	30.1
22.07.94	20.97	15.4	28.5	17.25	21.3	15.4	16.11	15.1	17.1	21.51	15.3	29.0
23.07.94	21.45	12.2	33.7	18.90	18.3	12.5	15.95	12.9	16.9	20.51	13.3	34.2
24.07.94	21.07	15.7	32.5	18.23	22.5	15.1	16.81	15.3	17.0	20.98	15.9	33.1
25.07.94	20.44	13.3	30.3	16.25	22.1	13.0	15.25	13.0	17.0	19.87	13.4	32.5
26.07.94	18.98	15.7	22.7	15.30	17.1	14.9	15.40	14.2	16.2	19.50	15.9	24.0
27.07.94	19.56	16.4	26.0	16.30	17.8	14.6	16.28	14.2	16.5	18.21	16.4	28.0
12.08.94	18.60	12.9	22.7	18.65	22.7	12.2	17.91	11.9	22.0	18.60	12.9	22.7
13.08.94	13.74	10.1	21.3	13.44	23.1	9.7	12.46	9.3	16.4	13.74	10.1	21.3
14.08.94	12.54	8.6	20.3	12.42	22.7	8.2	11.41	7.5	15.4	12.54	8.6	20.3
15.08.94	14.23	9.0	23.8	13.75	23.8	9.0	12.95	9.0	17.5	14.01	9.0	23.8
16.08.94	15.03	10.4	22.0	15.01	20.0	10.8	14.27	10.8	17.8	15.03	10.4	22.0
17.08.94	13.44	11.5	16.4	13.11	19.6	10.8	11.78	10.1	13.6	13.44	11.5	16.4
18.08.94	15.61	10.1	22.7	15.55	22.4	10.1	14.61	9.3	21.7	15.61	10.1	22.7
19.08.94	17.52	17.1	19.2	17.70	20.3	17.5	17.51	17.1	21.7	17.52	17.1	19.2

Table D5

Temperature in Radio-tracking Enclosure - 1995

DATE	Pasture			Rough Grassland			Woodland			Arable		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
17.07.94	18.26	14.0	25.2	18.08	14.3	21.0	17.31	14.0	21.0	17.97	12.9	42.2
18.07.94	21.37	14.0	40.9	18.87	14.0	24.9	17.32	13.6	20.3	21.27	13.6	32.1
19.07.94	25.13	16.1	38.0	22.76	17.1	27.1	20.23	17.5	23.4	22.85	14.7	37.2
20.07.94	26.91	14.0	43.9	23.53	15.7	30.0	20.30	16.4	25.2	24.48	12.6	36.8
21.07.94	20.88	9.3	37.2	18.77	11.5	26.5	16.50	12.6	19.2	20.61	7.9	40.5
22.07.94	18.73	7.5	45.3	16.78	9.3	31.1	14.19	10.8	18.2	18.81	6.0	49.2
23.07.94	22.00	6.0	48.2	18.49	10.1	33.7	15.37	10.4	20.7	21.73	4.9	49.2
24.07.94	22.39	9.0	40.5	22.97	11.1	38.4	19.92	11.5	29.2	24.89	7.9	43.8
25.07.94	26.23	12.0	43.8	24.50	16.0	33.8	21.30	17.0	23.8	25.89	14.0	42.0

Table D6

Humidity in Radio-tracking Enclosure - 1994

DATE	Pasture			Rough Grassland			Woodland			Arable		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
30.06.94	71.06	52.7	90	93.23	60.4	100	99.04	52.1	101	73.8	60.7	87
01.07.94	62.14	13.6	100.9	95.07	60.4	101	86.96	65.4	101	63.91	14.8	95.2
02.07.94	80.58	29.7	102	85.99	70.7	101	99.99	99	101	92.95	85.9	100
03.07.94	75.16	26.8	100.9	89	60.6	101	100.01	89	100.8	75.16	64	86
04.07.94	75.79	21.2	100.9	85	74.4	101	100.01	89	100	75.9	41	98
05.07.94	93.24	86.1	101	95	78.4	100	99.39	94.8	89.4	85	86	100
06.07.94	74.17	53.7	86.1	91	72.4	92.6	90.79	73.4	83.9	73	64	83
14.07.94	68.54	21.2	101	91.78	60.4	100	98.78	70.1	101	52.46	17.4	92.8
15.07.94	82.4	46.9	100	97.53	90	101	97.53	89	102	77.66	20.1	100
16.07.94	69.82	21.2	100	93.94	60.4	101	95	62	101	71.46	21.5	100
17.07.94	71.05	17.4	103	92.74	47	100	93	51	101.8	73.86	14.4	100
18.07.94	71.76	12.4	103	94.48	50.6	101	92	53	101.9	71.42	15.4	100
19.07.94	76.26	32.6	103	95.05	68.6	101	97	67	101.9	72.9	24.3	100
20.07.94	72.02	17.4	101	94.11	50.6	101	93	52	101	69.86	14.4	100
21.07.94	77.77	26.8	100	96.41	77.2	101	96	78	101	75.6	36.7	101
22.07.94	68.88	18.6	100	92.11	60.6	102	93	62	101	67.29	14.4	101
23.07.94	70.48	17.4	100	92.25	57	99.9	82.51	81	102	71.17	14.4	100
24.07.94	85.01	35.7	103	97.01	81.6	101	94.78	81	103	83.33	22.8	101
25.07.94	85.32	38.7	100	97.48	68.6	102.3	94.66	70.1	101	80.08	25.8	100
26.07.94	96.43	88.1	100	99.94	99	102	99.59	90	101	95.84	83.6	101
27.07.94	96.43	63	100	99.9	64	101	87.83	62.1	100	95.15	62.4	100
12.08.94	74.87	56.4	99	73.38	70.4	100	91.19	89.5	101	76.87	56.4	99
13.08.94	89.95	70	100	90.92	70.6	100	92.7	78	101	95.21	70.6	100
14.08.94	88.59	64.8	100	89.17	60.1	100	92.41	78	102	97.14	74.8	102
15.08.94	89.08	69.2	100	92.97	73.3	100	92.5	79	101	97.28	79.2	102
16.08.94	88.53	53	100	96.24	75	100	92.46	82	102	95.95	80	102
17.08.94	79.03	36	100	94.48	62.4	100	91.83	71	102	86.05	46	100
18.08.94	75.44	21.9	100	97.46	85	93.4	91.28	87.7	101	79.86	21.5	100
19.08.94	80.69	52.4	85.2	97.57	96	93.4	91.19	89.5	100	90.69	62.4	95.2

Table D7

Humidity in Radio-tracking Enclosure - 1995

DATE	Pasture			Rough Grassland			Woodland			Arable		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
17.07.94	77.98	62.4	100	78.52	55.8	55.8	88	67.1	67.1	75.97	50.7	99
18.07.94	95.95	77.2	101	88.8	48.6	48.6	99.67	97	97	86.75	45.3	102.5
19.07.94	79.2	40.1	100	77.39	43.5	43.5	97.64	77.5	77.5	77.68	42	100
20.07.94	72.69	17.4	100	78.41	40.9	40.9	89.4	63	63	77.68	20	100
21.07.94	74.67	27.3	100	85.21	52.2	52.2	98.31	93.1	93.1	74.87	29	102
22.07.94	65.59	16.1	99	78.16	36.9	36.9	86.4	65	65	75.67	18	101
23.07.94	64.78	16.1	100	79.85	40.2	40.2	86.25	61.1	61.1	71.39	18	101
24.07.94	61.66	20.1	98	76.87	46.4	46.4	81.68	44.2	44.2	75.58	22.1	103
25.07.94	47.93	38.1	72.5	67.15	54.2	54.2	74.45	52.4	52.4	55.2	33.5	86.7
26.07.94	55.15	47.1	76.4	60.4	51.5	51.5	62.81	47.1	47.1	75.76	47.1	100
27.07.94	57.02	50.7	66.2	65.5	53.4	53.4	66.62	63.4	63.4	67.87	51	82.7
28.07.94	61.4	35	97.9	74.66	51.5	51.5	77.91	61.5	61.5	68.69	38	100
29.07.94	58.96	7.6	100	75.89	38.9	38.9	79.23	51.5	51.5	75.82	9	101
30.07.94	58.48	7.6	99	73.95	37.3	37.3	77.27	49.6	49.6	72.12	10	101
31.07.94	61.42	9.9	100	74.54	38.9	38.9	74.47	38.9	38.9	70.73	9.9	100
01.08.94	77.32	12.3	100	90.08	47.8	47.8	89.61	47.8	47.8	79.93	12.3	100
02.08.94	62.76	9.9	100	74.5	40.7	40.7	73.4	40.7	40.7	72.08	12	102
03.08.94	62.65	6.6	101	76.07	44	44	72.49	34	34	77.84	10	103
04.08.94	62.58	8.8	100	74.8	44.2	44.2	74.63	44.2	44.2	71.84	12	101
05.08.94	61.18	7.6	100.4	73.41	42.4	42.4	72.24	42.4	42.4	76.94	12	100
06.08.94	68.64	11.1	101	82.59	51.5	51.5	83.09	51.5	51.5	75.29	12	101
07.08.94	70.51	27.3	98	82.3	59.1	59.1	82.4	59.1	59.1	76.61	27.3	100
08.08.94	64.04	6.6	100.4	72.89	44.2	44.2	75.12	51.5	51.5	65.93	6.6	101
09.08.94	63.49	7.6	100	74.17	42.4	42.4	76.92	52.4	52.4	74.91	15	100
10.08.94	60.59	6.66	101	68.91	35.6	35.6	74.77	45.6	45.6	72.34	10	100
11.08.94	59.04	5.4	101	70.66	32.3	32.3	74.26	42.3	42.3	71.63	15	100
12.08.94	62.14	17.4	100.4	80.16	51.5	51.5	77.86	51.5	51.5	73.84	17.4	101
13.08.94	67.17	18.7	101	80.7	53.4	53.4	80.6	53.4	53.4	75.19	19	101
14.08.94	67.99	14.8	99	81.8	55.2	55.2	81.07	55.2	55.2	73.76	22.8	101
15.08.94	65.58	12.3	101	80.36	51.5	51.5	80.72	51.5	51.5	74.15	14	101
16.08.94	64.52	9.9	101	77.2	44.2	44.2	77.17	44.2	44.2	77.39	10	100
17.08.94	66.23	6.6	101	75.36	35.6	35.6	75.1	35.6	35.6	73.49	9	100
18.08.94	70.28	17.4	100	84.44	44.2	44.2	80.6	44.2	44.2	74.07	17.4	100

APPENDIX E

E1 Phase I Habitat Survey Methods

The habitat surveys in chapter 7 are based on the Phase I habitat surveys. Habitats were viewed and areas of seemingly homogenous habitat (e.g. deciduous woodland, cereal crop) were marked on the OS map. The nature of each habitat was assessed by eye, using the rules described in Table E1. The categories shown in Table E1 are based on Phase I NVC surveys with some modifications to adapt the categories to the Leicestershire area which is dominated by farming and industry.

Table E1

Definitions Of Habitat Categories And Procedure For Identification

Habitat Category	Definition	Procedure
Woodland	A habitat dominated by trees greater than 5m high when mature, with more than 10 trees shading two x 25m cross transits.	Two 25m transits at 90° to each other were walked. The number of trees which were deciduous and those which were coniferous were counted. All trees with any above ground parts directly crossing the transits were counted.
Sub categories	Deciduous Coniferous Mixed Recently felled	>90% Deciduous >90% Coniferous
Scrub	A habitat dominated by woody perennials less than 5m when mature.	Scrub which was too dense to walk through was put in this category. If possible the number of woody perennials in two x 25m cross transits were counted as described for woodland.

Table E1(cont..)
Definitions Of Habitat Categories And Procedure For Identification

Grassland	A habitat dominated by species of the *Gramineae* Except Usually less than 30 cm sward height	The sward height was measured with a ruler in the centre of five 2m x 2m quadrats. The presence of a litter component was recorded. The dominant species were identified. Dominant species were those with the highest percentage coverage (estimated by eye).
Sub categories	Unimproved short Unimproved long Hay meadow Improved Amenity Improved short Ley	< 10cm sward height > 10 cm sward height Identified from farmers and litter component Short (<10cm sward height) , mowed <10 cm sward height improved Ephemeral planted grasslands
Tall Herb and Fern	Vegetation dominated by non-woody perennials which were not cultivated. Taller than 20 cm in height.	Same procedure as for grassland. Note the percentage cover is estimated as the cover of above ground parts of the plants.
	Marsh Bracken Species rich vegetation Tall ruderal	Dominated by *Juncaceae* and *Cyeraceae* species > 50% coverage Bracken No single species greater than 30% coverage
Boundary	Linear land features less than 5m wide.	Length of linear features was marked on map.
Sub categories	Hedgerow Hedgerow with trees Fence Wall Ditch Earth bank	Woody perennials, in a linear row, no individuals reaching maturity. As for hedgerow but some individual trees reached maturity

Table E1(cont..)
Definitions Of Habitat Categories And Procedure For Identification

Habitat Category	Definition	Procedure
Cultivated	Any area tilled or ploughed on a regular basis.	Identified dominant crop in two 2m x 2m quadrats. Crops and managements also identified by enquiry of farmers and land owners
Sub categories	Arable Cereal Arable Non-cereal	
Built-up Areas	Areas dominated by non-vegetation/ non-industrial features	The following were included in this category Buildings, large areas of concrete, roads.
Rock Exposure and Waste	Any bare ground dominated by rock or resultant from industrial activity	Identify activity by enquiry of land managers. Walk the site
Sub categories	Bare ground Quarry Spoil Mine Rubbish tip	>80% Rock
Open Water	Any area of permanent water.	Perimeters were walked and measured by pacing and direct measurement with tape where possible.
Sub categories	Standing water Running water	This was the breeding site

Table E3

Population Indices And Breeding Pond Descriptions.

Pond Number	O.S. Grid Reference	Population Index 1994		Population Index 1995		Breeding Site Description
		M	F	M	F	
1	SK393147	*	*	48	6	Farm Pond for cattle.
2	SK402044	4	1	86	11	Farm Pond for horses.
3	SK426026	23	4	130	13	Large rural garden duck pond.
4	SK437027	128	41	446	62	Conservation pond on quarry site.
5	SK361227	969	243	1932	564	Large fishing lake connected to reservoir on national trust site.
6	SK356237	2	1	17	3	Small fish pond on national trust site.
7	SK465153	649	209	672	172	Disused reservoir on nature reserve.
8	SK437183	217	91	283	73	Fishing lake in farmland.
9	SK427151	264	83	402	66	Boating lake in suburban park.
10	SK439107	16	4	20	7	Fishing lake in brickworks.
11	SK437106	3	1	5	0	Ornamental pond in brickworks.
12	SK413121	62	9	150	18	New nature reserve.
13	SK399185	651	88	554	94	Farm fish pond.
14	SK407165	512	83	617	98	Re-landscaped open cast mine (old).
15	SK502153	217	39	394	56	Private fish pond in large rural garden
16	SK516134	79	8	146	11	Farm pond for sheep.
17	SK405065	4	1	18	3	Arable farm pond.
18	SK388174	23	10	67	8	Disused pond in pharmaceutical company grounds.
19	SK382126	13	2	27	5	Farm pond for horses.
20	SK385122	1	0	8	3	Arable farm pond.
21	SK475162	96	22	189	25	Farm pond for ducks and cattle.
22	SK425047	754	150	856	73	Fishing lake of private residence and farm.
23	SK475179	170	24	526	91	Filled in quarry near housing estate.
24	SK380210	75	14	135	21	Re-landscaped open cast mine (new).
25	SK387188	533	205	317	46	Fish lake in grounds of hospice.
26	SK423179	10	1	56	16	Fishing lake by suburban residences.

Population indices are the numbers of animals before correction for clear water and transformation. M = Peak Count of Males and F = Peak Count of females. * = no population estimates were obtained in this year.

Table E4(a)

Areas Of Each Habitat Category Less Than 500m Radius From The Breeding Site

Pond Number	AREA OF HABITAT CATEGORIES (TRANSFORMED)							
	Arable	Boundary (length)	Garden	Long Grassland	Short Grassland	Urban/ Industrial	Water	Woodland
1	30.5	4.1	4.2	0.0	49.6	0.0	2.7	0.4
2	28.8	4.1	12.3	0.0	51.4	18.5	4.8	26.7
3	28.0	4.9	18.6	0.0	56.0	10.6	4.9	6.6
4	36.2	3.8	8.5	11.6	48.0	11.2	9.7	9.9
5	0.0	0.0	3.0	19.7	31.7	0.0	13.0	51.5
6	20.6	6.1	20.8	7.2	47.6	0.0	4.2	29.6
7	17.2	4.8	0.0	47.1	25.3	15.3	5.3	24.8
8	35.2	5.0	0.0	11.5	43.7	12.5	5.9	24.9
9	29.0	4.6	18.3	26.3	42.0	8.2	14.7	0.0
10	33.0	2.4	14.6	13.9	32.9	31.4	11.9	0.0
11	23.4	2.4	22.4	14.0	41.1	26.7	4.4	9.4
12	45.7	4.8	21.5	24.4	28.0	0.0	8.8	0.0
13	49.1	5.2	10.1	30.6	20.5	7.5	4.2	6.3
14	22.4	3.3	0.0	13.6	36.3	0.0	9.1	44.1
15	22.7	3.9	4.6	15.4	54.5	10.2	6.0	20.6
16	28.6	5.0	5.0	3.9	47.0	0.0	3.2	31.6
17	49.9	4.1	7.9	0.0	38.1	5.6	2.1	4.7
18	43.7	2.3	0.0	0.0	32.3	5.1	7.9	31.5
19	46.5	3.9	12.3	0.0	40.0	0.0	0.0	4.5
20	38.6	4.1	0.0	0.0	37.2	12.5	0.0	0.0
21	18.5	5.5	5.3	14.7	57.8	0.0	3.7	23.4
22	50.6	4.7	15.8	28.0	9.4	12.5	8.1	15.8
23	26.1	4.4	0.0	9.6	43.4	14.4	0.0	4.8
24	17.4	0.0	0.0	0.0	35.1	36.1	4.7	30.2
25	28.9	3.3	12.2	16.9	12.4	0.0	18.0	46.4
26	41.9	4.6	19.9	28.4	24.5	11.0	3.1	14.5

Variables are the arcsine transformations of the percentage area of each habitat type within a 500m radius of the breeding site.

Table E4(b)

Areas Of Each Habitat Category Greater Than 500m Radius From The Breeding Site

Pond Number	AREA OF HABITAT CATEGORIES (TRANSFORMED)							
	Arable	Boundary (length)	Garden	Long Grassland	Short Grassland	Urban/ Industrial	Water	Woodland
1	51.5	4.4	0.0	0.0	28.1	7.7	0.0	22.6
2	37.6	5.0	14.0	0.0	42.9	9.3	0.0	16.3
3	41.3	5.8	6.1	0.0	46.5	6.5	0.0	7.0
4	45.7	5.0	12.3	0.6	37.8	4.4	1.6	13.4
5	19.8	5.0	0.0	9.5	41.3	0.0	7.0	35.6
6	18.9	4.9	17.8	6.6	45.9	3.6	6.8	26.4
7	10.9	5.9	2.9	30.2	42.4	7.5	0.0	25.8
8	43.6	5.9	8.4	3.7	31.8	6.0	1.6	24.9
9	39.4	5.6	16.0	8.0	40.0	8.9	0.0	3.6
10	50.8	4.8	0.0	0.0	27.0	12.5	2.8	6.0
11	50.0	4.8	22.7	0.0	19.1	22.2	6.6	0.0
12	36.4	6.6	16.0	3.1	39.1	22.1	0.0	3.1
13	44.2	6.7	15.1	7.4	35.9	8.5	1.7	4.4
14	21.1	4.8	18.6	4.0	46.7	1.8	0.0	25.2
15	34.7	5.4	8.3	7.1	44.8	9.5	0.0	20.3
16	24.7	5.4	3.0	8.3	41.3	7.0	3.5	32.3
17	51.2	5.3	9.1	0.0	35.2	7.5	1.6	7.5
18	24.0	5.3	0.0	2.2	53.8	0.0	3.1	33.8
19	40.5	5.1	1.3	7.0	39.3	0.0	2.4	3.1
20	41.0	6.1	5.0	2.5	36.6	1.8	0.0	2.6
21	28.3	5.3	3.0	15.1	35.9	10.9	0.0	29.2
22	48.7	5.7	1.3	5.9	35.0	0.0	0.0	7.9
23	45.1	5.4	16.1	2.6	40.2	5.7	0.0	10.0
24	7.3	2.1	0.0	0.0	34.3	43.7	0.0	23.4
25	41.8	5.3	5.1	11.0	31.0	0.0	7.4	23.4
26	27.4	5.8	25.3	0.0	43.3	7.3	0.0	20.2

Variables are the arcsine transformations of the percentage areas of each habitat category from 500 to 1000 m radius from the pond